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Personalized Approach in Acute and Chronic Leukemias

Emmanuel C. Besa, M.D.
Professor of Medical Oncology
Hematologic Malignancies

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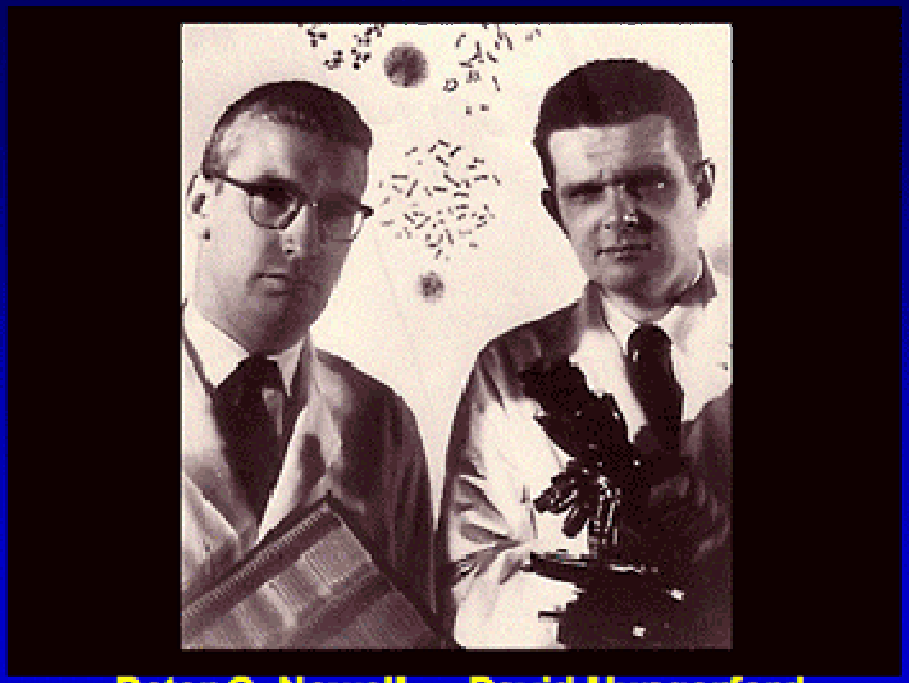
The Perfect Storm in Hematologic Malignancy in Acute and Chronic Leukemias

- Discovery of the Philadelphia Chromosome as a specific diagnostic marker led to its molecular mechanism in Chronic Myelogenous Leukemia (CML)
- Translocation led to discovery of BCR-ABL translocation gene which proved to be pathogenetic fusion protein (tyrosine kinase)
- Designed tyrosine kinase inhibitor (TKI) imatinib blocks specific protein results in reversing CML at the molecular level
- Change the natural course of the disease resulting in possible cure and eliminating risky bone marrow transplantation as only curative therapy.

Legacy of the Philadelphia Chromosome Discovery

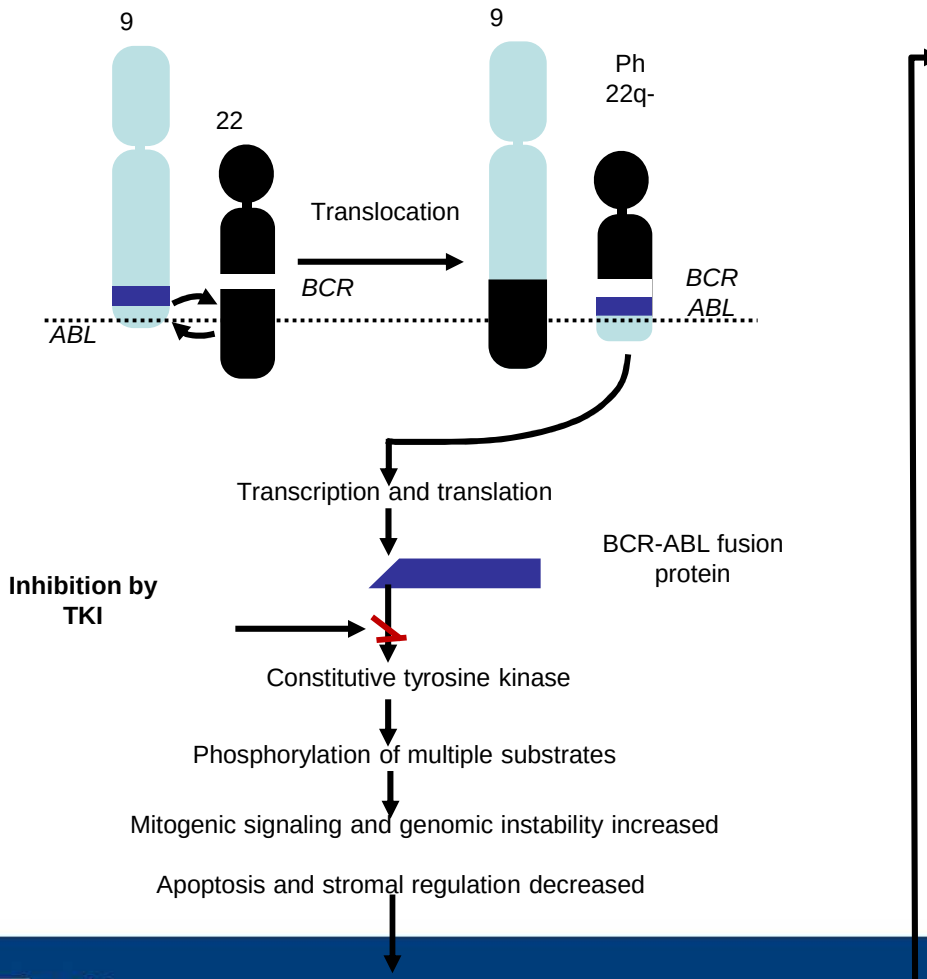
1960: Philadelphia-chromosoom



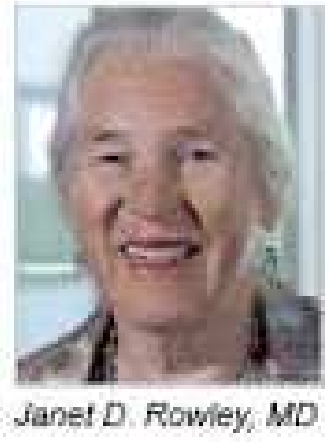
Peter C. Nowell David Hungerford

Winnaars van de Nobelprijs

Philadelphia Chromosome Translocation in CML Results in BCR-ABL Oncogene



- Stem cell disorder
- Characterized by myeloproliferation
- Well-described clinical course



Janet D. Rowley, MD

Natural Course of CML

Accumulation of immature myeloid cells
New cytogenetic changes

	Chronic Phase	Accelerated Phase	Blast Phase
Duration	If untreated, 3-5 yrs	Varies	Median survival of several mos
Prognosis	Responsive to treatment	Decreased responsiveness	Resistant to treatment
Symptoms	Asymptomatic OR Fatigue Weight loss Abdominal pain or discomfort Night sweats	Progressive splenomegaly Myelofibrosis	Bleeding complications Infection complications

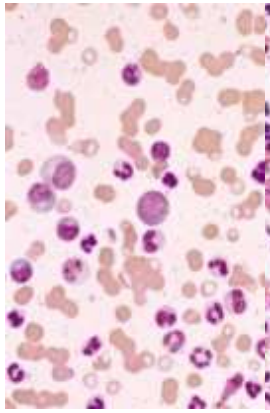
Radich JP, et al. Proc Natl Acad Sci U S A. 2006;103:2794-2799. Sawyers CL. N Engl J Med. 1999;340:1330-1340. Druker B, et al. Chronic leukemias. In: Cancer, principles, and practice of oncology. 17th ed. 2005.

Diagnosis of CML

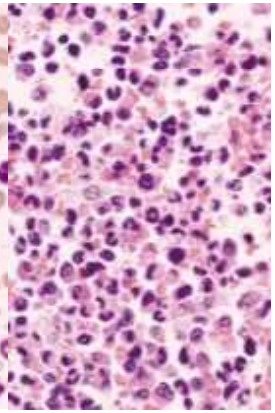
Sensitivity

Hematologic | **Cytogenetic** | **Molecular**

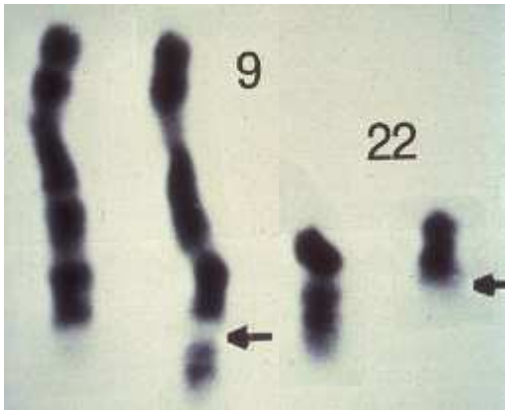
Karyotype (Ph chromosome) | FISH | PCR (BCR-ABL fusion)



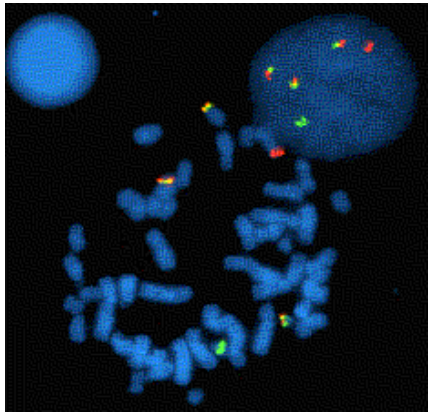
Peripheral blood
(with myeloid cells)



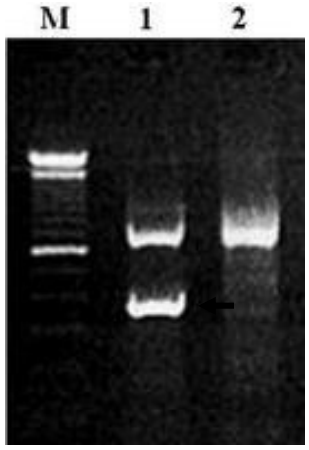
Bone marrow
(with myeloid hyperplasia)



Chromosomal translocation
t(9;22)(q34;q11)



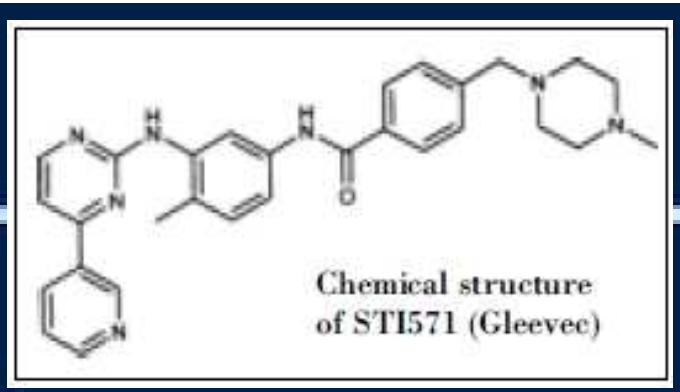
Abnormal BCR-ABL
Red: BCR
Green: ABL
Yellow: fusion



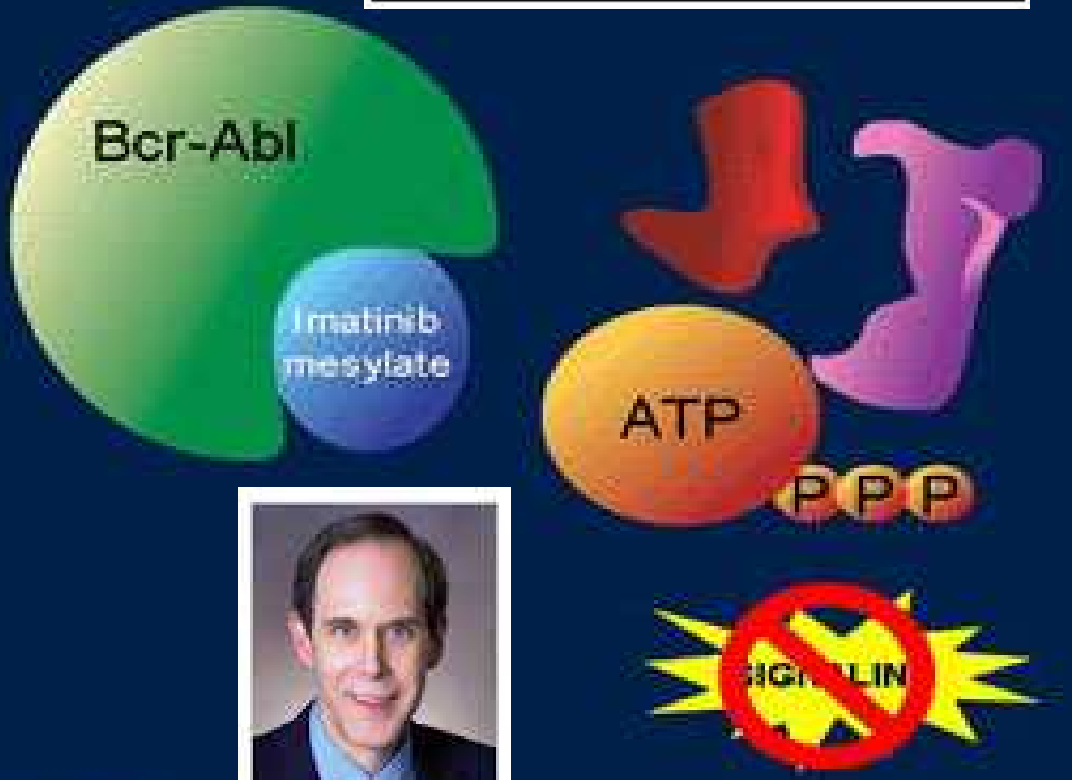
Abnormal BCR-ABL
Lane 1: BCR-ABL+
Lane 2: BCR-ABL-

Criteria for Hematologic, Cytogenetic, and Molecular Response

Imatinib Mesylate: Mechanism of Action



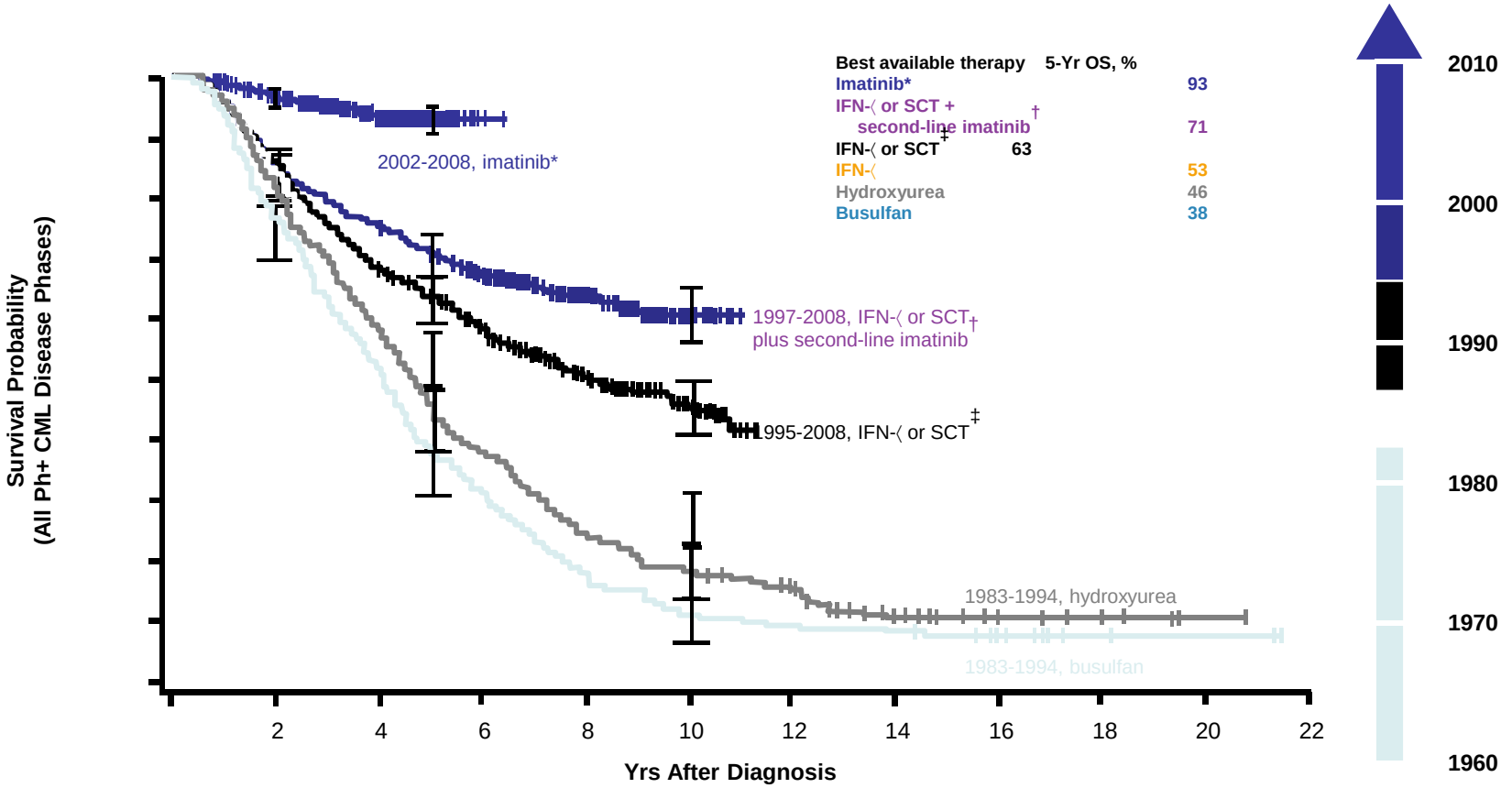
- Imatinib mesylate occupies the ATP binding pocket of the Abl kinase domain
- This prevents substrate phosphorylation and signaling
- A lack of signaling inhibits proliferation and survival



Brian J. Druker, MD

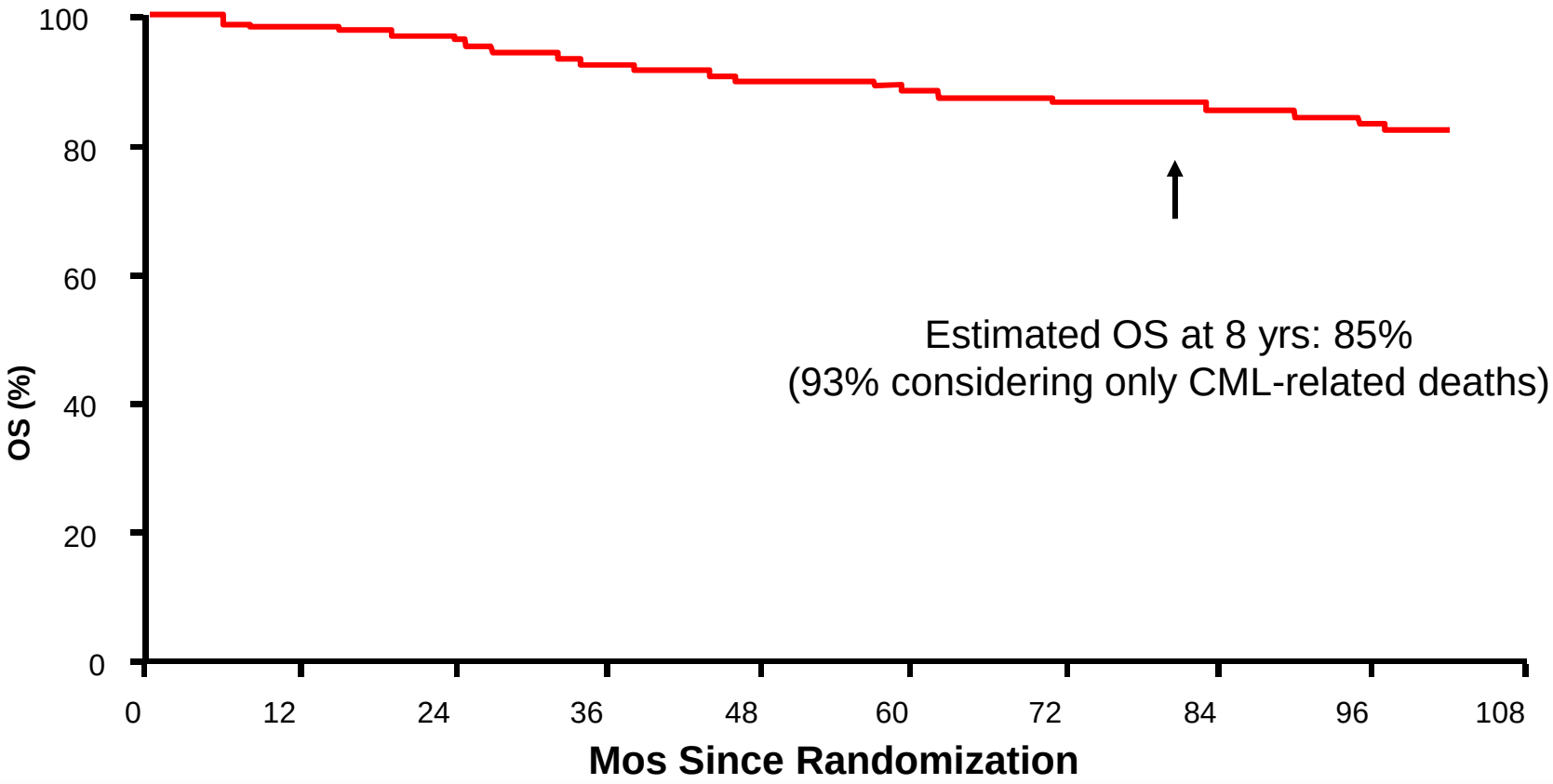
Savage DG, Antman KH. *N Engl J Med*. 2002;346:683-693.

Imatinib Changed the Therapeutic Landscape for Patients With Ph+ CML



*CML IV. †CML IIIA. ‡CML III.

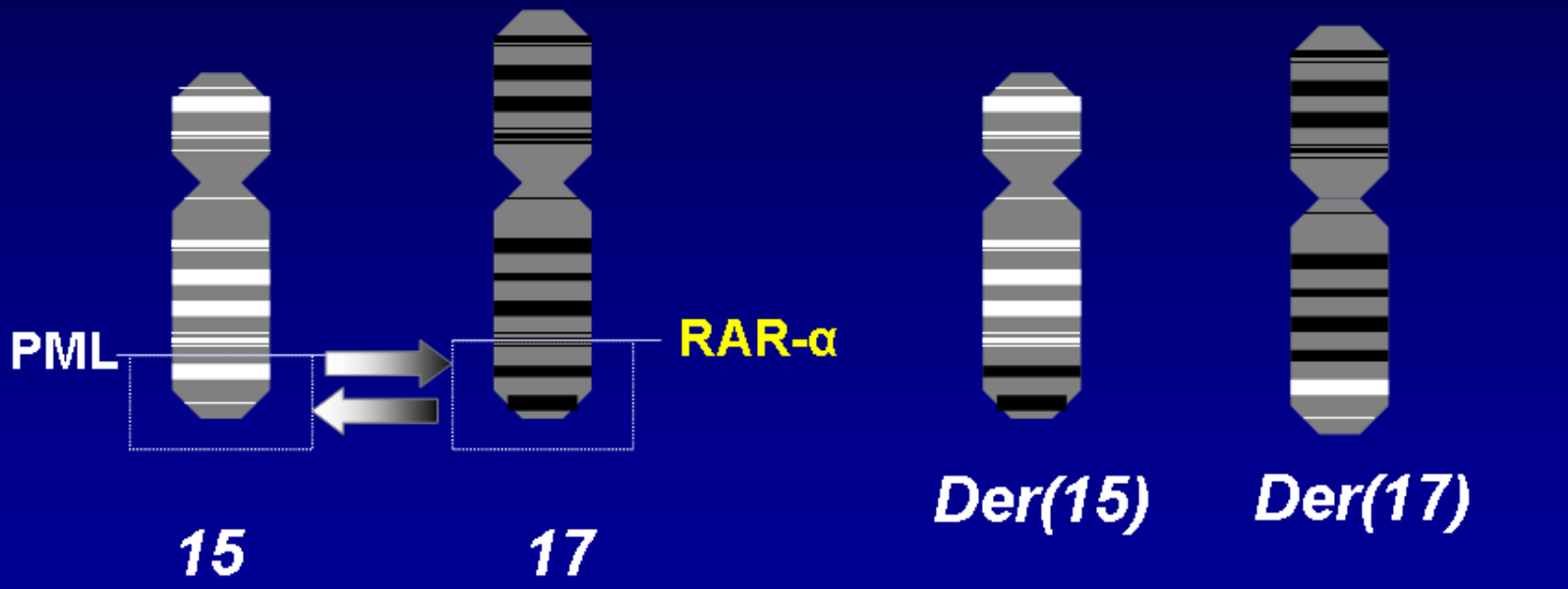
IRIS 8-Yr Update: OS (ITT) With Imatinib Treatment in CML



Deininger M, et al. ASH 2009. Abstract 1126.

APL Chromosomal Changes

t(15;17)(q22;q11-22)

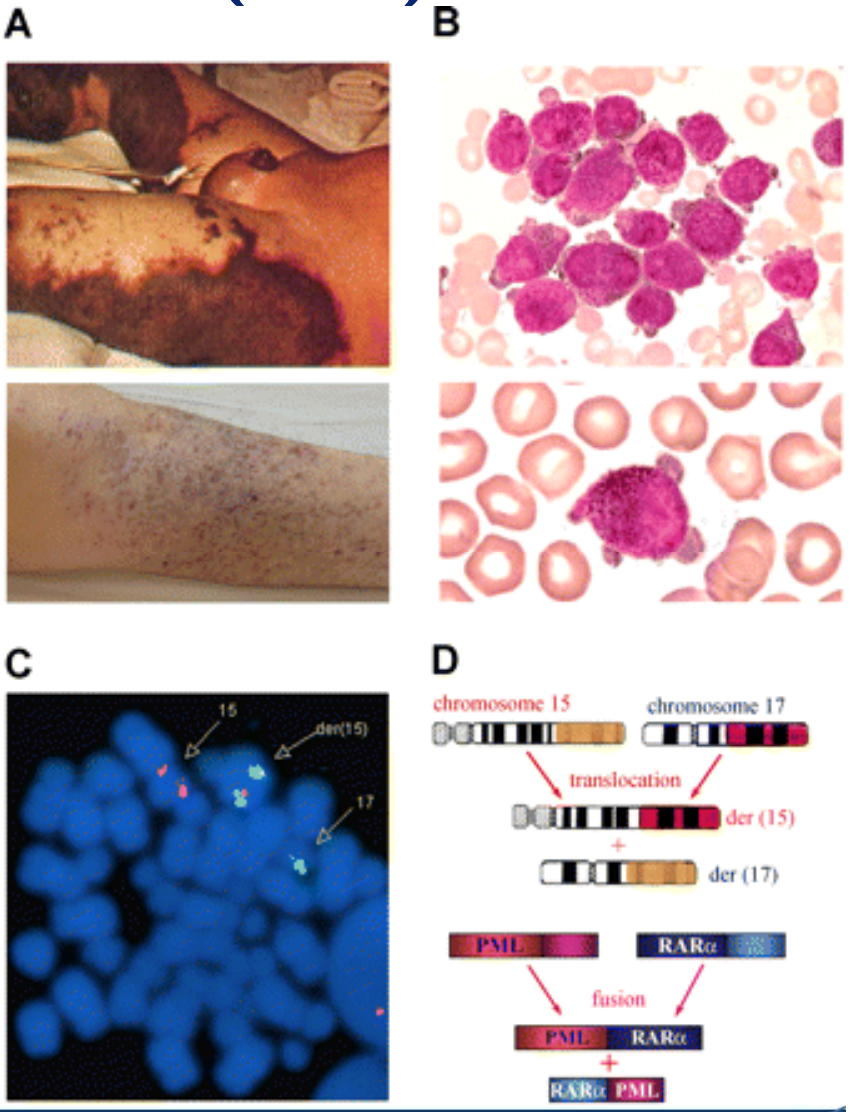


Cytogenic Methods of Confirming APL

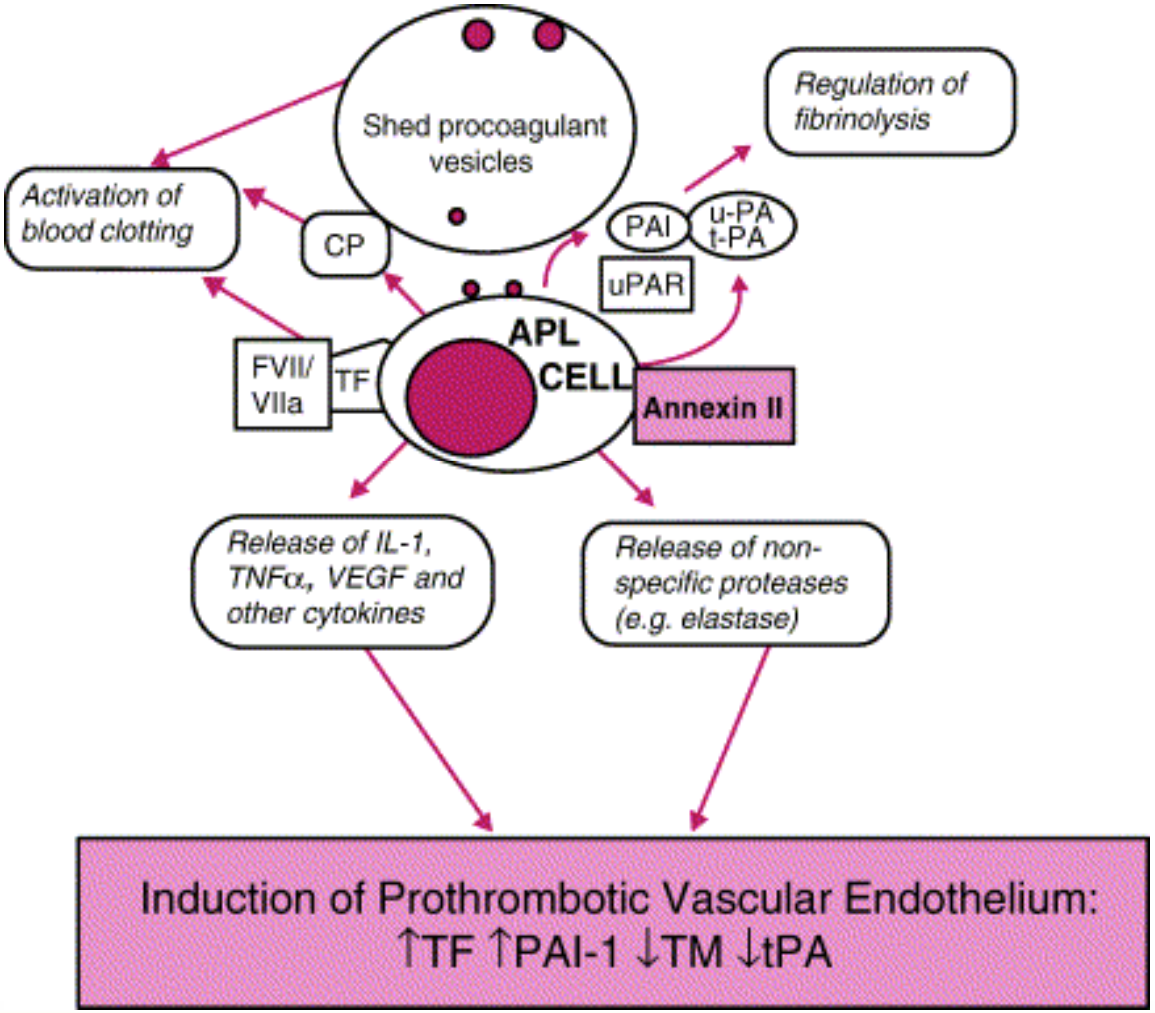
- PML/RAR-α fusion protein plays a role in pathogenesis of APL
- Test to establish a diagnosis of APL include:
  - Cytogenetics
  - Fluorescence *in situ* hybridization (FISH)
  - Reverse transcriptase polymerase chain reaction (RT-PCR)

Acute Promyelocytic Leukemia (M-3)

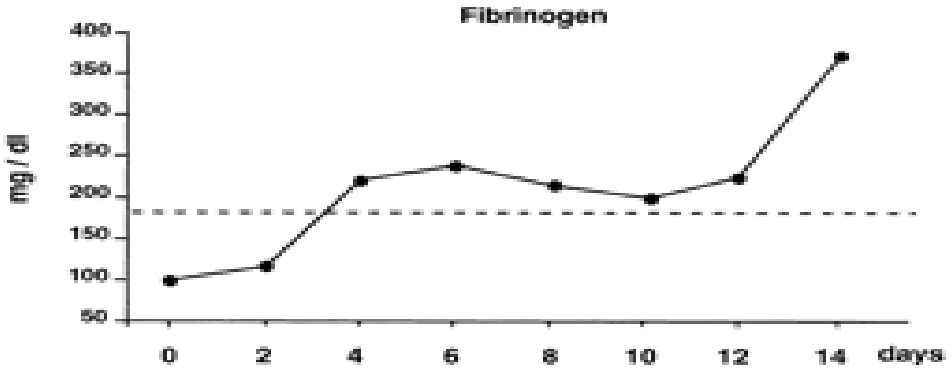
- A) a severe bleeding tendency due to fibrinogenopenia and disseminated intravascular coagulation
- (B) accumulation of abnormal promyelocytes in bone marrow and chromosomal translocation t(15;17)(q22;q21)
- (C) with the resultant fusion transcripts between PML and RAR detected by FISH using *PML-RAR* dual-color, dual-fusion translocation probes
- D) Schematics representing the formation of 15;17 reciprocal chromosomal translocations and fusion transcripts

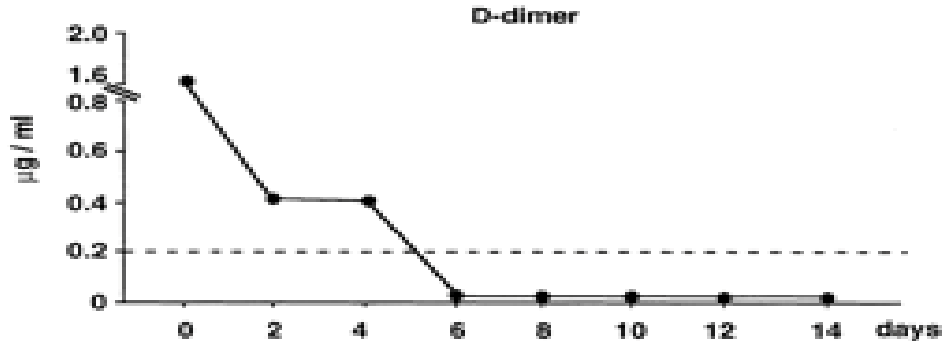


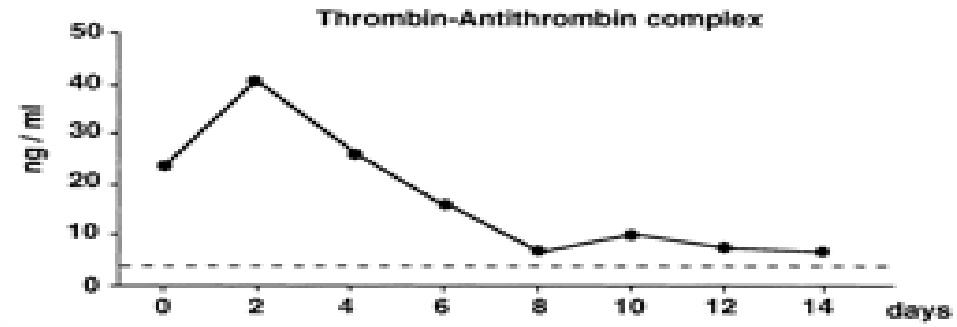
Mechanism of DIC in APL



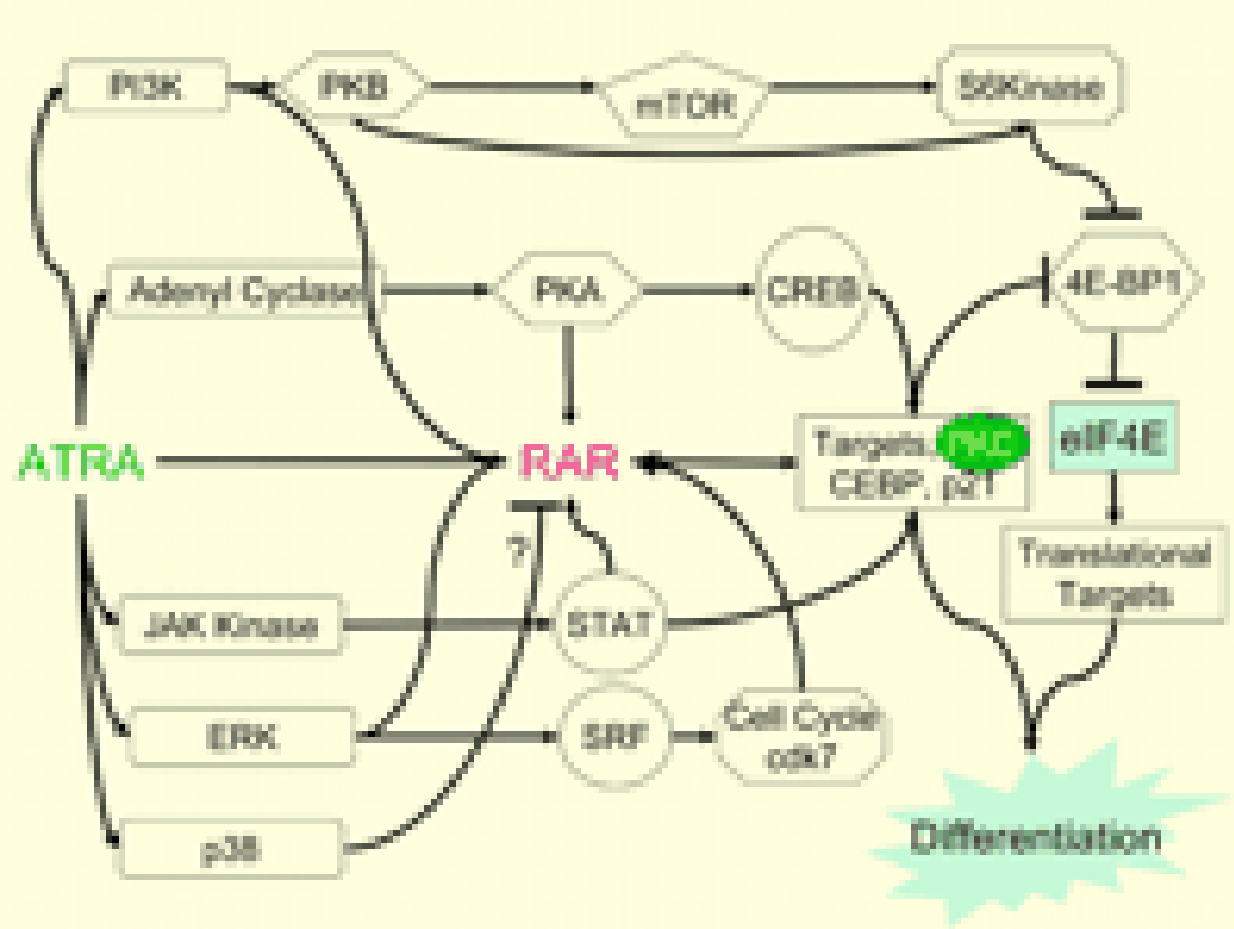
Reversal of Coagulopathy by differentiation with ATRA or ATO



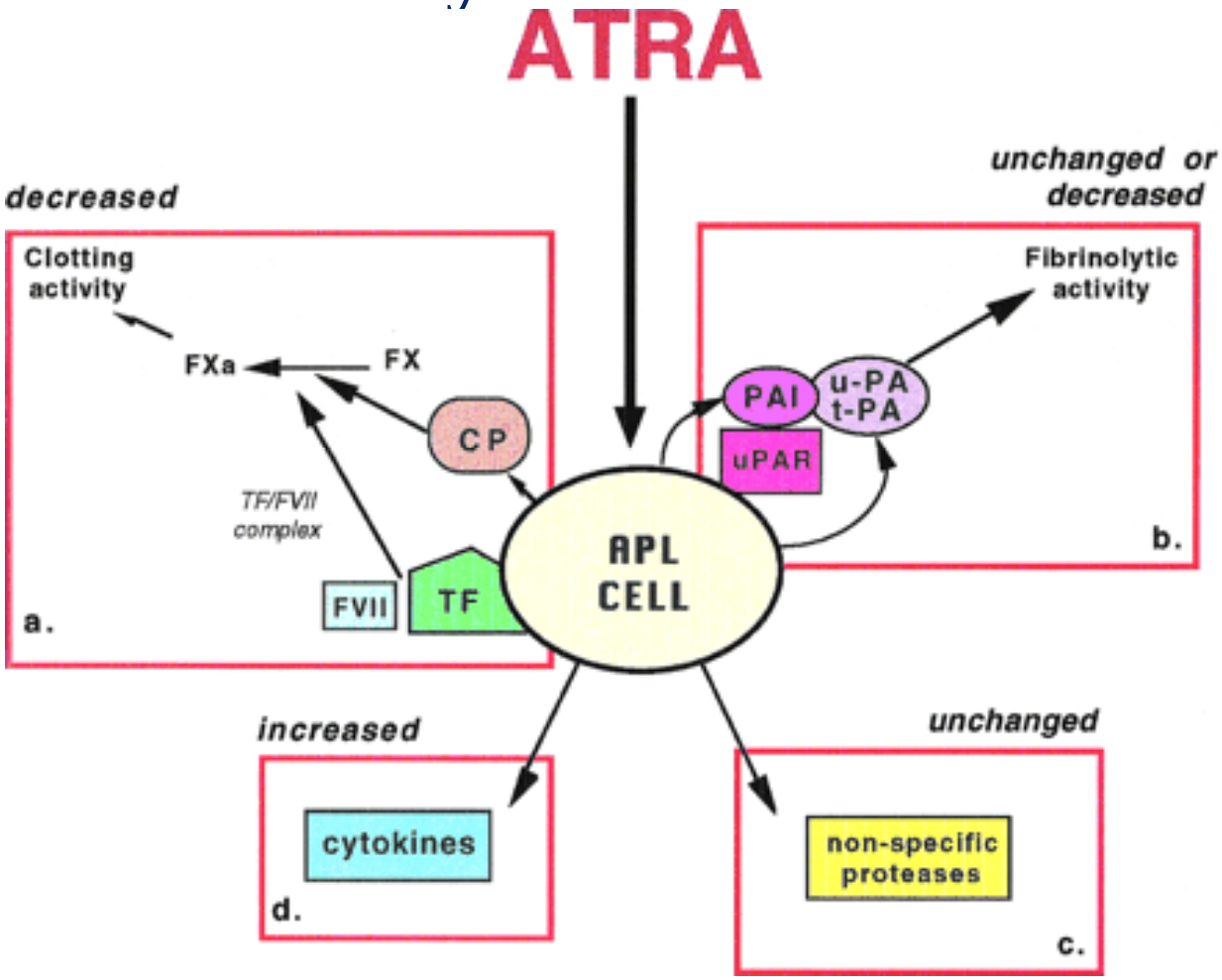




Mechanism of Action ATRA



Mechanism of ATRA for reversal of DIC & induce Retinoid Syndrome



Differentiation Syndrome due to Increased Cytokines (Cytokine Storm)

• High WBC counts as leukemic cells differentiates promotes expressions of one or more adhesion molecules with high incidence of vascular complications and early mortality

• Clinical features of Retinoid syndrome are:

- unexplained fever,
- weight gain,
- respiratory distress,
- interstitial pulmonary infiltrates,
- pleural and pericardial effusions,
- episodic hypotension
- acute renal failure.

Special supportive care during induction of APL

- Once APL is suspected by morphology or presence of DIC the patient should be treated as an emergency.
- Start ATRA and supportive measures even before the molecular diagnosis of PML-RARa is available to lower risk of life-threatening hemorrhage.
- Reversal of on-going coagulopathy should be based on liberal Tx of FFP and fibrinogen to keep levels >1.5 g/L (150 mg/dL)
- Thrombocytopenia should be treated with aggressive platelet support by keeping platelet counts > 30-50,000/µl until coagulopathy resolves.
- Awareness of the APL differentiation syndrome which are weight gain, SOB, increasing WBC leading to ARDS which is life-threatening by starting early in course dexamethasone 10 mg bid IV. Do not stop ATRA unless life-threatening and adding chemotherapy does not help.

Ref: Sanz MA. ASH Educ Book Hematology 2006, page 147.

ATRA as a differentiation therapy for APL: first model of targeted therapy

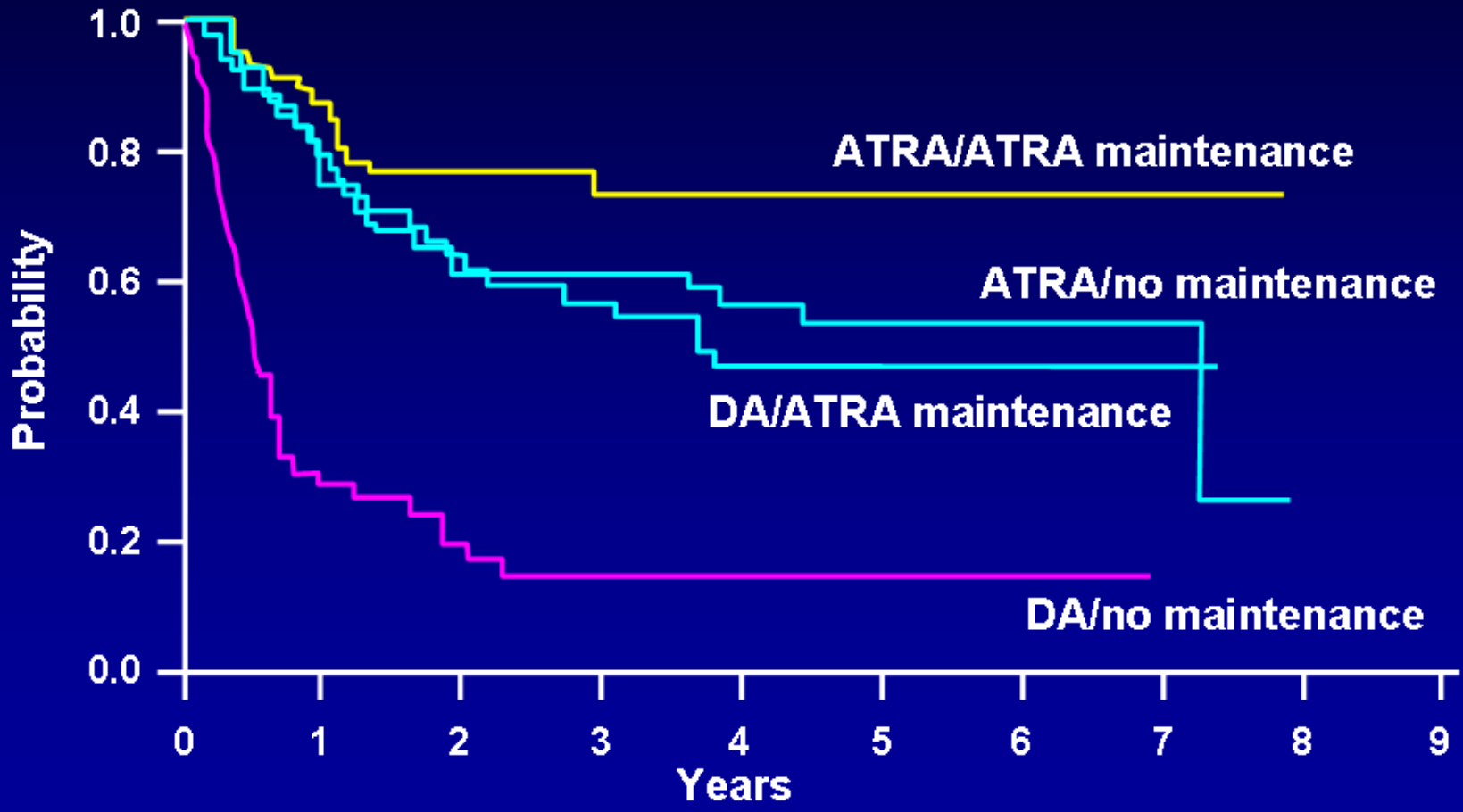
- Chinese medicine based on Confucius philosophy of guiding bad elements instead of killing them led to differentiation approach to AML supported by shift in Western medicine.
- In vitro evidence of cellular differentiation of leukemic cells by retnoic acid and ATRA was better than 13 CRA
- First APL patient treated with ATRA was a 5-year-old girl who received medical care in Shanghai Children's Hospital in 1985
- CR of 85% can be achieved in APL with ATRA alone, continuous treatment of APL with ATRA will cause progressive resistance to the drug and reduction of its plasma concentration because of accelerated clearance, resulting in relapse usually within 3 to 6 months requiring additional chemotherapy for cure.

ATRA- Chemo Combinations

- AIDA- ATRA + Idarubicin (GIMEMA)
- ATRA was administered orally beginning on the first day of induction at the dosage of 45 mg/m2/d until complete remission (CR), wheras IDA was administered intravenously at the dosage of 12 mg/m2/d on days 2, 4, 6, and 8 of the induction. Patients who achieved CR were consolidated with 3 courses of chemotherapy without ATRA; thereafter, they were followed up for molecular and hematologic CR
- The overall survival and event-free survival durations are 85% and 69% respectively; moreover, 14 of 18 (78%) patients who achieved a CR are still alive and in first molecular (RT-PCR) and hematologic CR.
- Although the majority of patients with APL are potentially cured by treatments combining all-trans retinoic acid (ATRA) and chemotherapy (CHT), a sizable proportion (around 30%) will relapse during follow-up.
- Ref: Diverio D et al. Blood 92:784, 1998

North American Intergroup Protocol Update 2002: Median Follow-up 6 Years

Relapse-free Survival



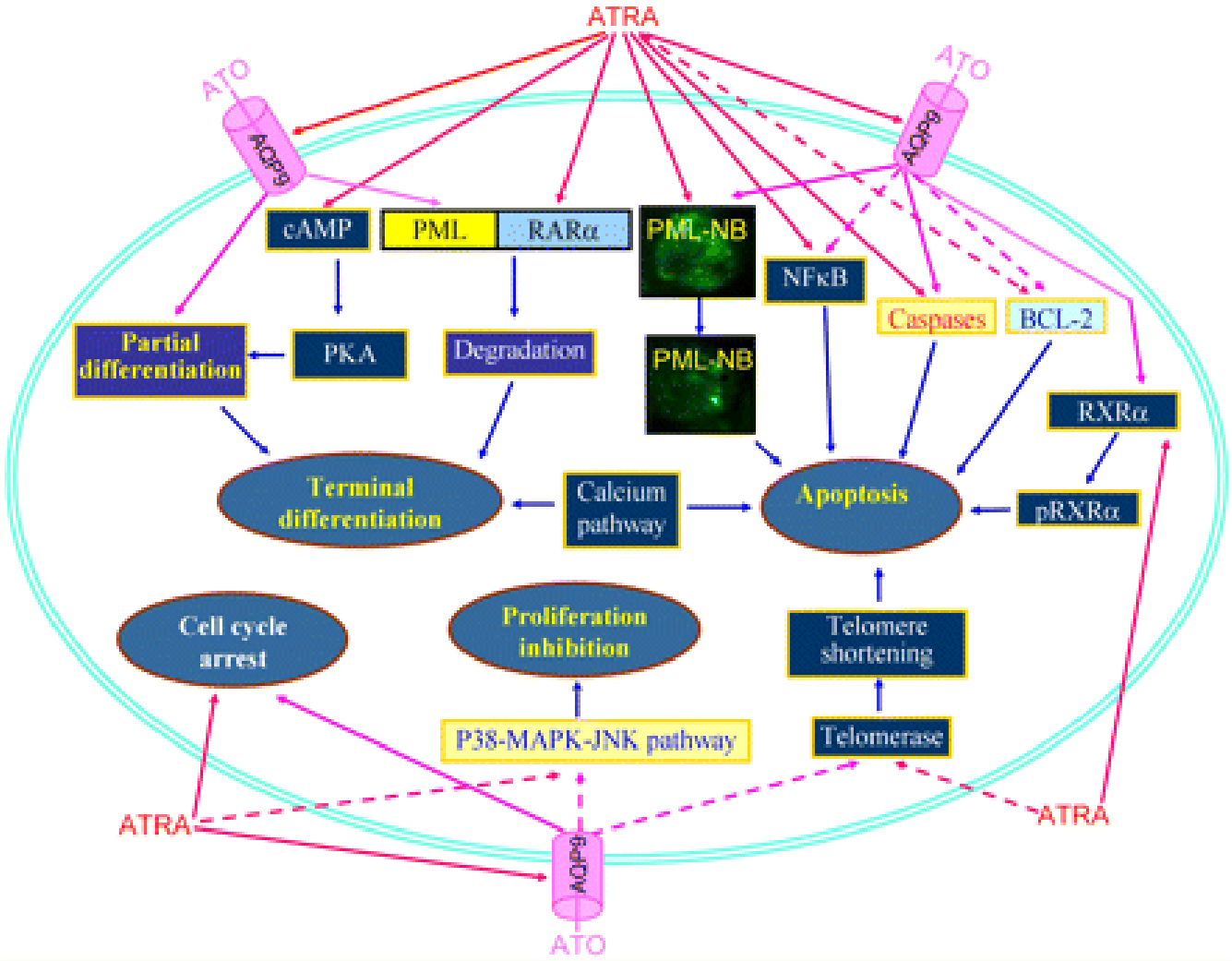
DA=daunorubicin + Ara-C; Ara-C=cytarabine

Tallman et al. *Blood.* 2002.

Use of ATO in the treatment of APL: taming an evil with a toxic agent

- Arsenic a common naturally occurring substance is one of the oldest drugs in both Western (Hippocrates, 460 BC) and Chinese (NeiJing, 263 BC) medicine for treatment of malaria and CML
- Sun et.al treated first APL with ATO resulting in 30% survival at 10 yr
- Multicenter studies show ATO as highly effective as a single agent resulting in long term remissions with 3 yr DFS of 87%
- AS4S4, another arsenic compound is also highly effective as shown by Lu et al (2002) show a 80% CR rates in newly diagnosed APL
- A composite natural Realgar-indigo tablet has a 98% CR rates in 60 APL patients
- Mechanism of action is different in that ATO exerts a dose-dependent activity; ie.differentiation in lower doses and apoptosis in higher doses thru mitochondrial apoptotic pathways
- Combining ATRA and ATO is synergistic

Schematic representing synergic/additive effects of ATRA and ATO.



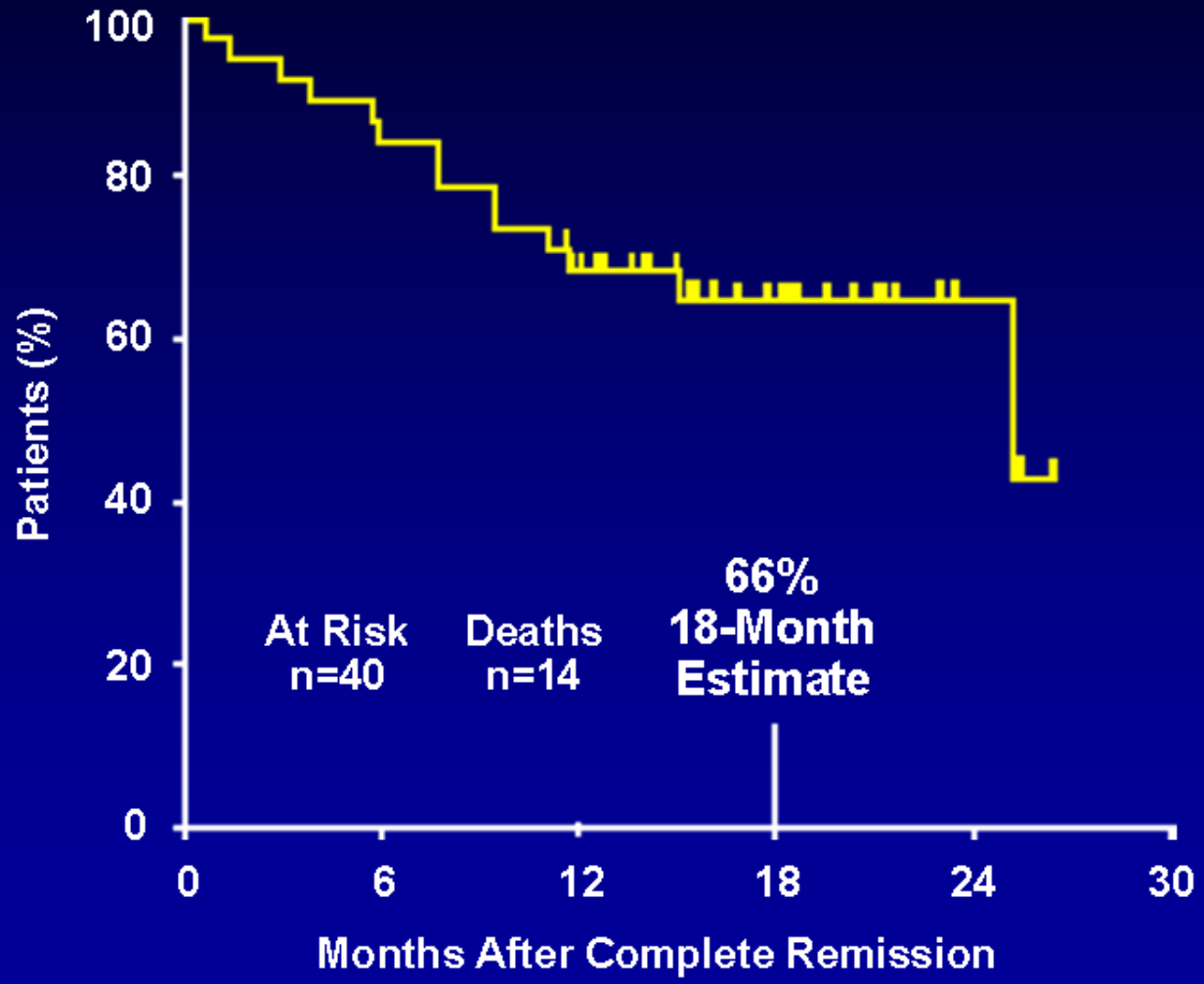
Oral Arsenic Trioxide ± Chemotherapy in Patients with Relapsed APL

- Treatment (N=50): oral AS_2O_3 10 mg/day until CR, ff by consolidation therapy w/ Idarubicin 6 mg/m2/d ove 3 months.
- Maintenance therapy (n=27): AS_2O_3 10 mg/d x 14 + ATRA

Response	Patients (N=50)
CR, n (%)	49 (98)
• Still in remission at median 61-mo ff-up, n	27
• Relapse after achieving CR, n	22*
• CR re-established in relapsed pts, n	19†
4-year OS, n	71
EFS, %	53

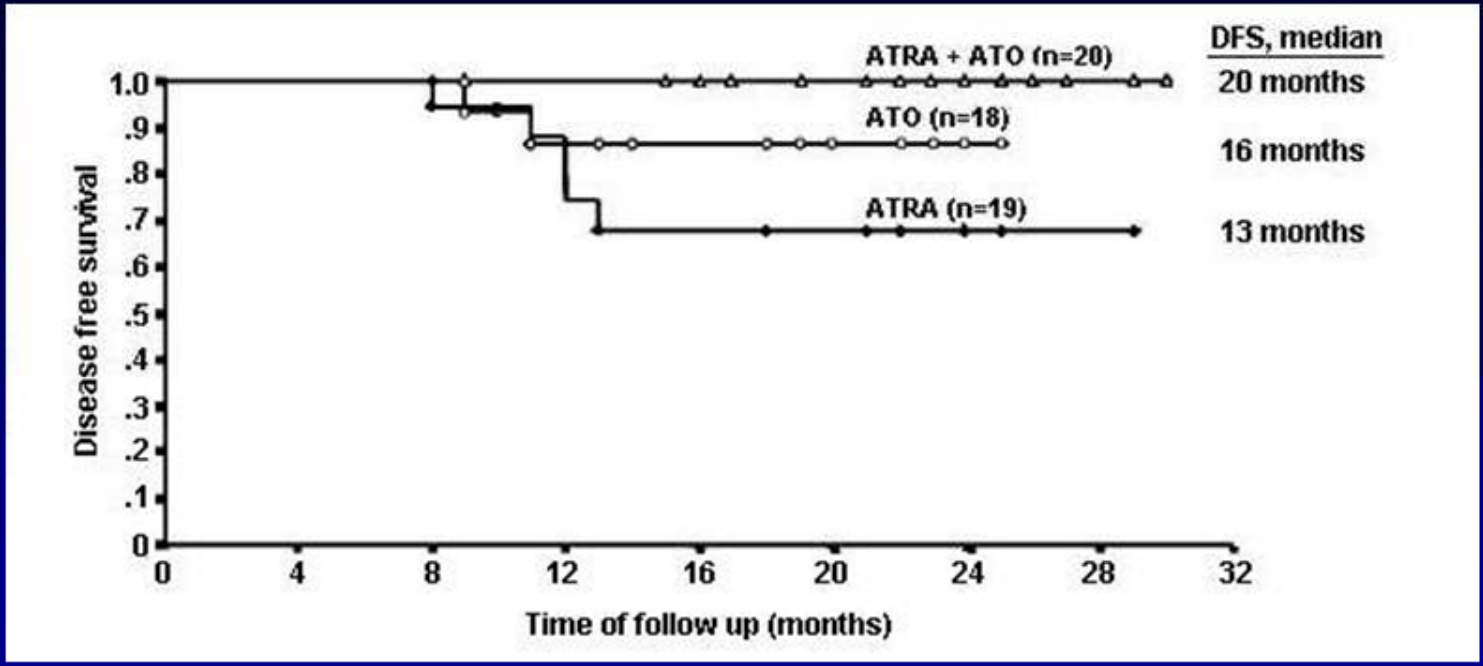
•*13 with maintenance, 9 without maintenance
•†8 remain in remission at median 88-month follow-up

US Multicenter Study: Overall Survival



Soignet, et al. *J Clin Oncol.* 2001.
TRISENOX Study PLRXAS01

Newly Diagnosed APL
ATO: Results (cont'd)

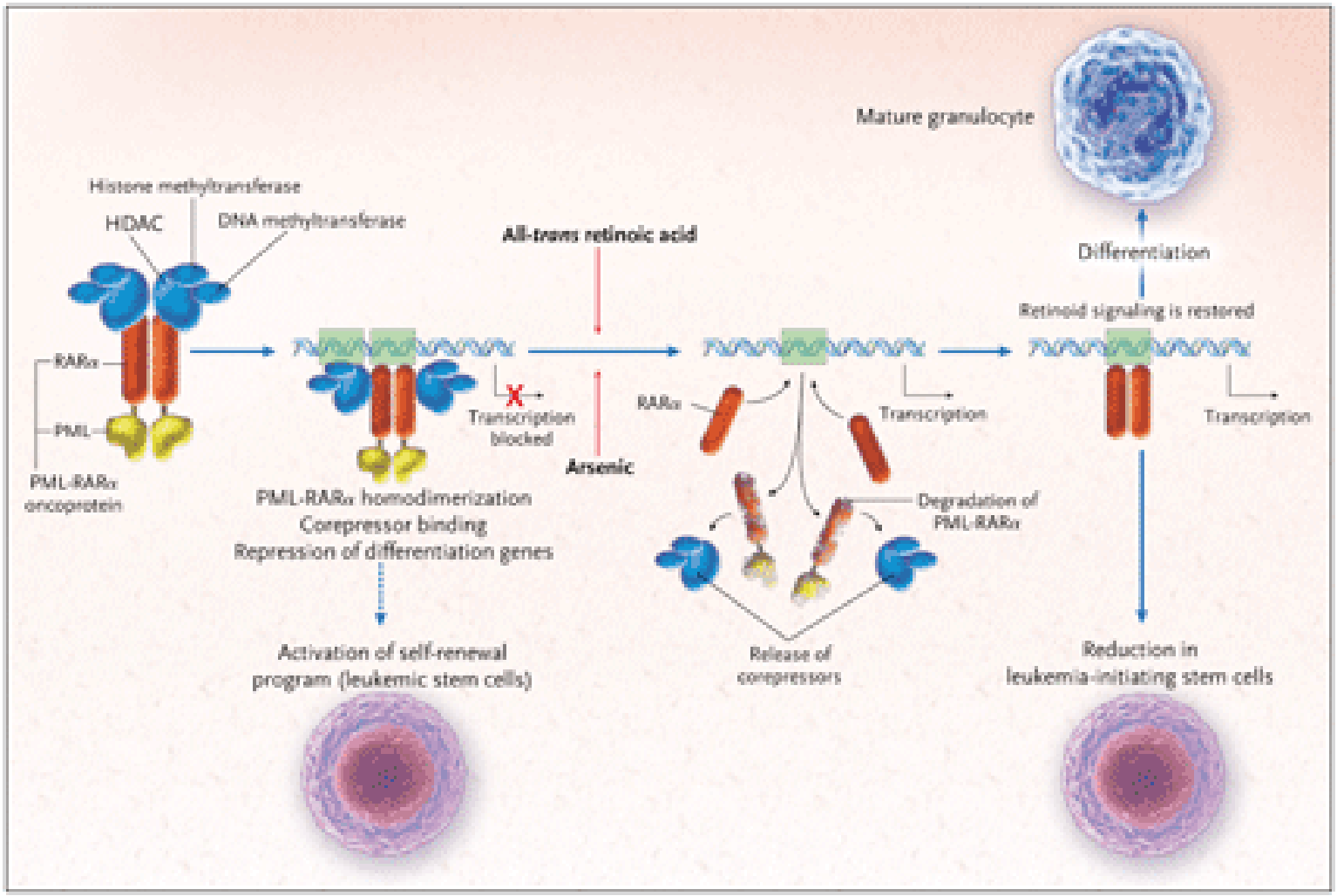


Source: Kaplan-Meier DFS survival curves adapted from Shen Z-X, et al. *PNAS*. 2004.

- The relapse rate of ATRA alone is significantly higher than that of ATRA combined with ATO (p=0.0202, Fisher's exact test). When patients of the two monotherapy groups are put together, the relapse rate is also statistically higher than that of ATRA and ATO combined (p=0.038)

ATO: arsenic trioxide; ATRA: all-*trans* retinoic acid; DFS: disease free survival Shen Z-X, et al. *PNAS*. 2004.

Countering an Oncoprotein



Core Binding factor (CBF) in AML Rx

- In primary adult AML, the highest CR rate (90%) and the longest DFS (50% at 5 yrs) have been associated with presence of CBF [t(8;21)(q22;q22) and inv(16)(p13q22) or t(16;16)(p13;q22)]
- Superior outcome of CBF gene rearrangement vs other AML may be attributed to increased sensitivity of blast cells to cytarabine and anthracyclines (backbone of AML Tx).
  - CALGB compared three consolidation using High (3 g/m2), Intermediate (400 mg/m2) or Low (100 mg/m2) Ara-C after first CR.
  - 7 yr ff up, show estimated patients in CR at 5 yrs was 42% after HDAC, 33% Intermediate DAC, 17% Low DAC consolidation.
  - The best results is using HDAC for consolidation in CBF AML.

Outcome Prediction in CBF AML using Gene Profile (CALGB Study)

- FLT3 mutations did not predict outcome in CBF AML
- Patients with predicted poor outcome in CBF AML had higher expression of genes with leukemogenic potential
  - WT1 in t(8;21) and inv(16)
  - CCNA1 in t(8;21)
  - MYCN in inv(16)
- Gene expression profiling improves outcome prediction among patients with CBF AML.
- Mutations in the KIT, FLT3, JAK2 and RAS genes, haploinsufficiency of the putative tumor suppressor genes TLE1 and TLE4

Ref: Paschka, P, eta. **Outcome prediction in adult core binding factor (CBF) acute myeloid leukemia (AML) with gene ex pression profiling: A Cancer and Leukemia Group B (B) study.** Abstract ASCO 2007.

Summary of Molecular Markers in AML

High Risk	Favorable Risk
FLT3/ITD mutations (high AR)	NPM (nucleophosmin) mutations
KIT mutations in CBF AML	CEBP〈 mutations
MLL PTD mutations	
Poor response to therapy (MRD) by flow	
High WT1, BAALC, ERG expression	
Gene expression profiles	Gene expression profiles

Role of genotype-based approach in the clinical management of adult AML with normal cytogenetics

- AML is the most common form of acute leukemia affecting adults.
- Nearly 50% of patients exhibit a normal karyotype (CN-AML) with an intermediate cytogenetic risk.
- Recurrence of genomic aberrations ie. mutations of FLT3, CEBPA, NPM1, RUNX1, TET2, IDH1/2, DNMT3A, ASXL1, MLL and WT1
- Provides novel insights into biology of this tumor, furnishes accurate prognostic markers as well as useful tools for selecting the most appropriate treatment option.
- Good prognostic markers such as CEBPA and NPM1 separates the good from rest of the intermediate group patients.
- The bad prognostic markers such as FLT3 suggest a poor response to standard therapy and justify more aggressive approach.

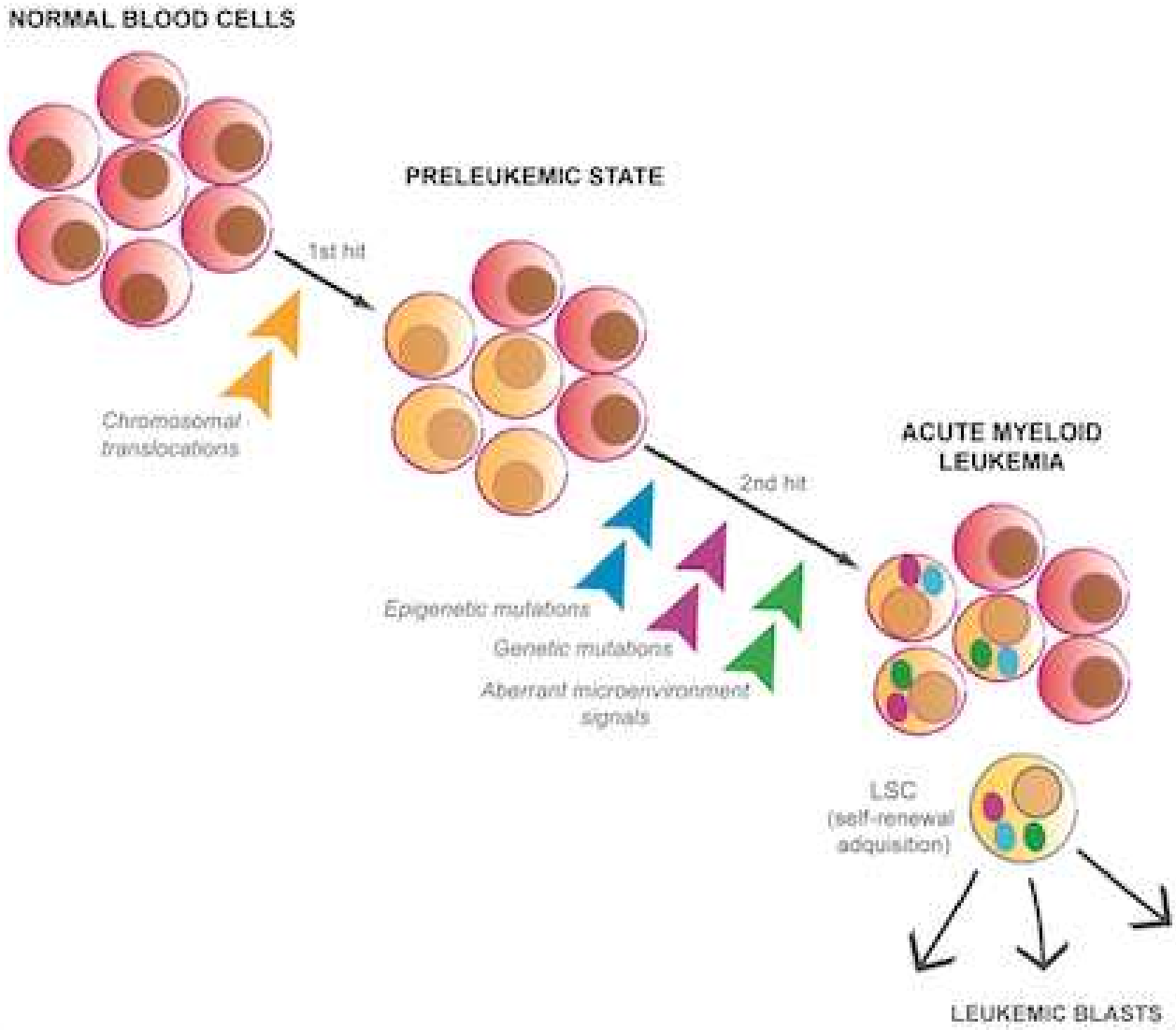
Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics

- 45% of adults with acute myeloid leukemia (AML) have normal cytogenetics and therefore lack structural abnormalities that can assist in the localization and characterization of molecular defects.
- The partial tandem duplication of the ALL1 (MLL) gene has been found in several such cases of AML, yet its frequency and clinical significance are unclear.
- Found in ~11% ± 5% of patients making it a frequent molecular defect with shorter duration of CR and thus require new therapeutic approaches.
- More studues are necessary to refine our treatment options in AML with normal cytogenetics.

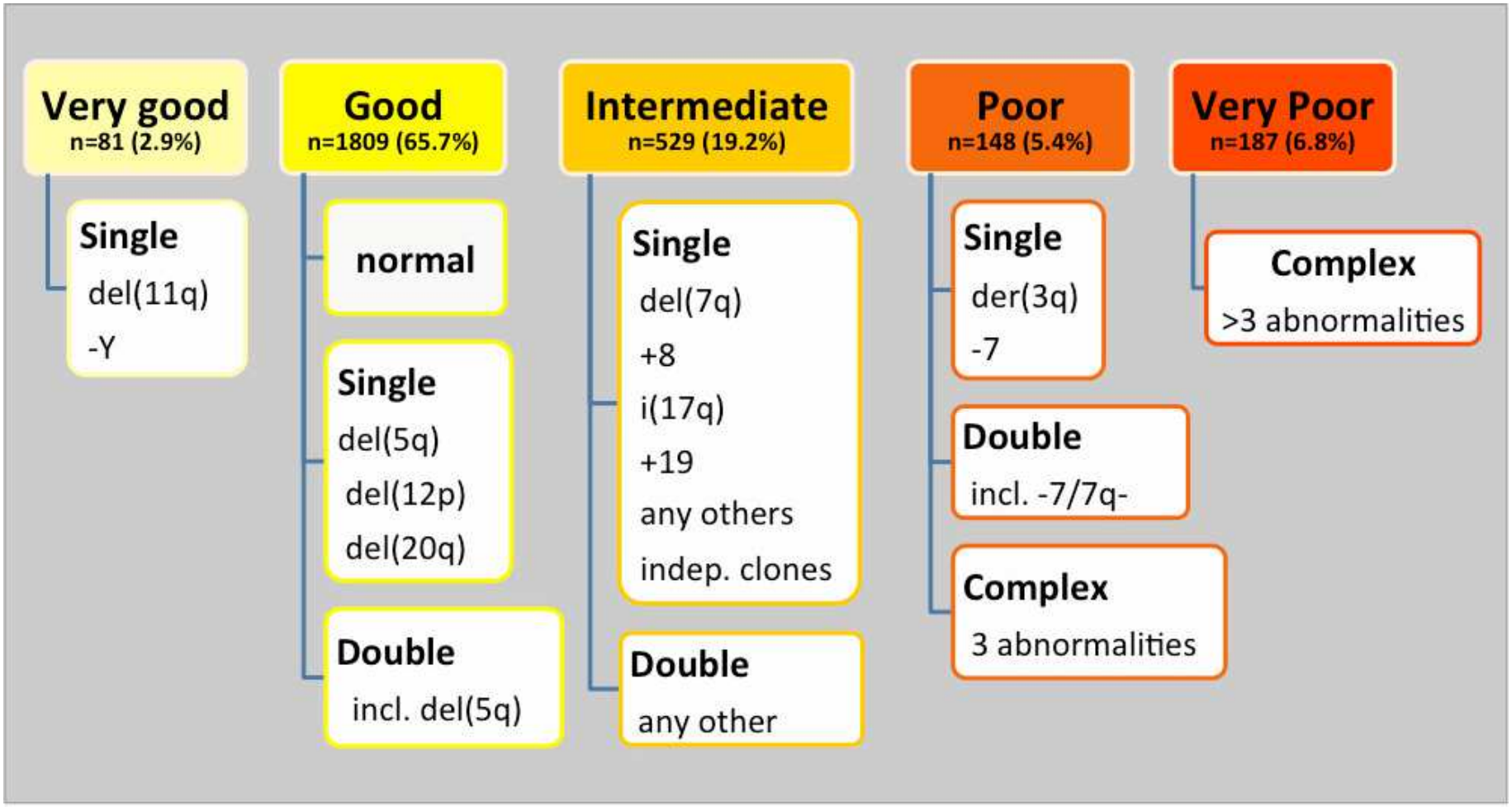
Combined Cytogenetic and molecular markers in AML

Risk Status	Cytogenetics	Molecular Abnormalities
Better	inv(16)[a] or t(16;16)[a] t(8;21)[a] t(15;17)	Normal cytogenetics, with *NPM1* mutation or isolated *CEBPA* mutation in the absence of *FLT3*
Intermediate	Normal +8 t(9;11) Other non-defined	t(8;21), inv(16), t(16;16): with c-KIT[c] mutation
Poor	Complex (three or more abnormal clones) –5, 5q–, –7, 7q– 11q23–non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[b]	Normal cytogenetics: with *FLT3*-ITD mutation[d] in the absence of *NPM1* mutation

Evolution of Acute Leukemia



Revised Survival of individual Cytogenetic in Preleukemic States such as Myelodysplastic Syndrome (MDS)



(Schanz J., et al., JCO (2012) Mar 10;30(8):820-9)

Frequency of Mutation and Association with Median Survival.

Table 1. Frequency of Mutation and Association with Median Survival.*

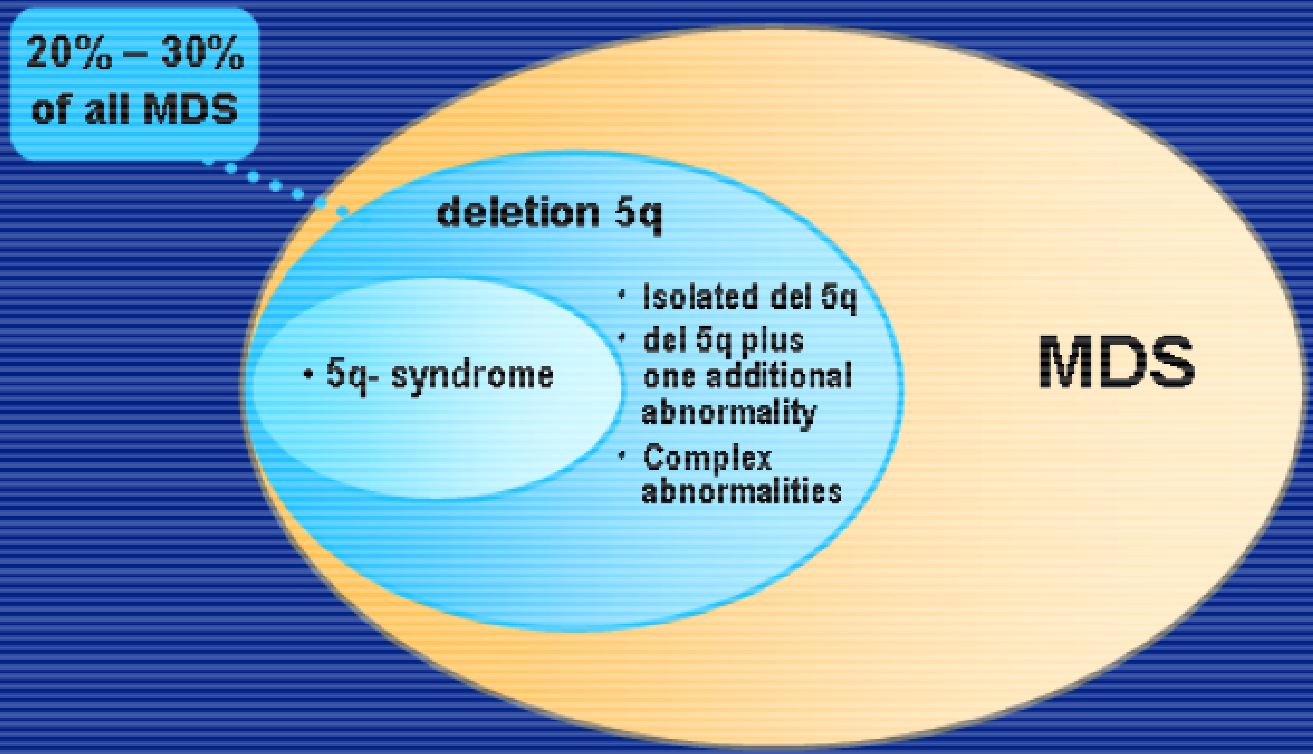
Mutated Gene	No. of Samples (%)	Median Survival (95% CI)	P Value
		yr	
All samples	439 (100)	1.86 (1.60–2.14)	
TET2	90 (20.5)	1.88 (1.26–2.55)	0.48
ASXL1	63 (14.4)	1.33 (0.96–1.88)	0.003
RUNX1	38 (8.7)	1.16 (0.77–1.53)	<0.001
TP53	33 (7.5)	0.65 (0.44–1.10)	<0.001
EZH2	28 (6.4)	0.79 (0.67–1.40)	<0.001
NRAS	16 (3.6)	1.03 (0.44–1.98)	0.006
JAK2	13 (3.0)	2.14 (1.02–3.12)	0.96
ETV6	12 (2.7)	0.83 (0.62–2.29)	0.04
CBL	10 (2.3)	1.52 (0.14–1.71)	0.02
IDH2	9 (2.1)	1.58 (0.50–2.14)	0.03
NPM1	8 (1.8)	2.18 (0.59–2.74)	0.43
IDH1	6 (1.4)	3.30 (0.35–9.52)	0.52
KRAS	4 (0.9)	0.89 (0.36–7.44)	0.54

Bejar R et al. N Engl J Med 2011;364:2496-2506.

Classes of somatically acquired driver mutation in MDS, MDS/MPN and MPN

Signaling	Epigenetic	mRNA Splicing	Transcription	Cohesin	Cyto
TKI fusions	*TET2*	*SF381*	*RUNX1*	*STAG2*	*5q-*
Jak2	*DNMT3A*	*SRF2*	*P53*	*SMC1A*	*-7/7q*
MPL	*IDH1/2*	*U2AF1*	*ETV6*	*SMC3*	*+8*
CBL	*EZH2*	*ZRSR2*	*BCOR*	*RAD21*	*+19*
NF1	*ASXL1*	*LUC7L2*	*CUX1*		*t(17q)*
NRAS	*PHF6*	*PPRF2*			*del(11q)*
KRAS	*CREBBP*				*del(12p)*
CSF3R	*EP300*				*Del(20q)*
RIT2					*Inv(3q)*
PTPN11					*t(3.3)*
FLT3					
KIT					
SETBP1					

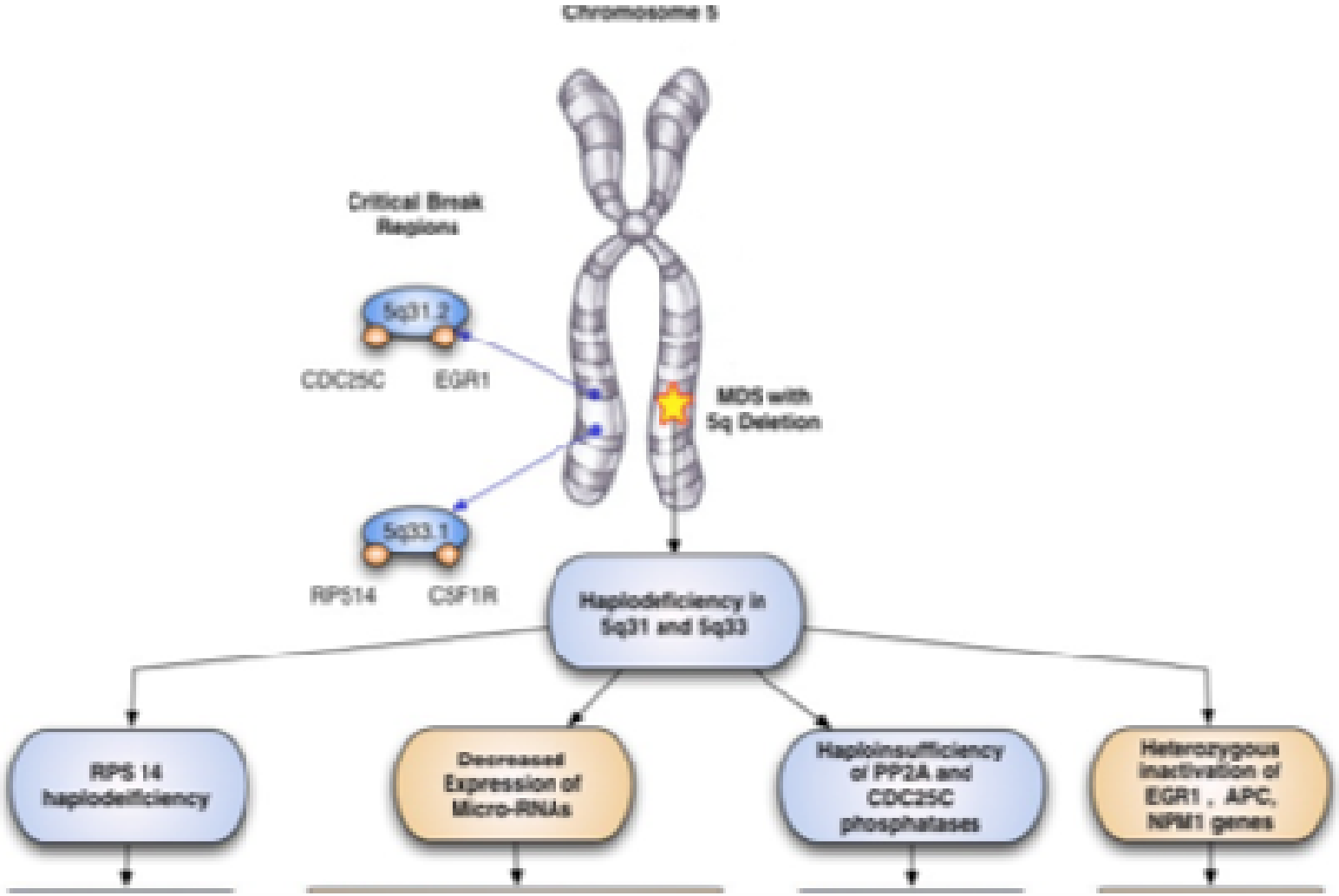
Deletion 5q is the Most Common Cytogenetic Abnormality Found in MDS



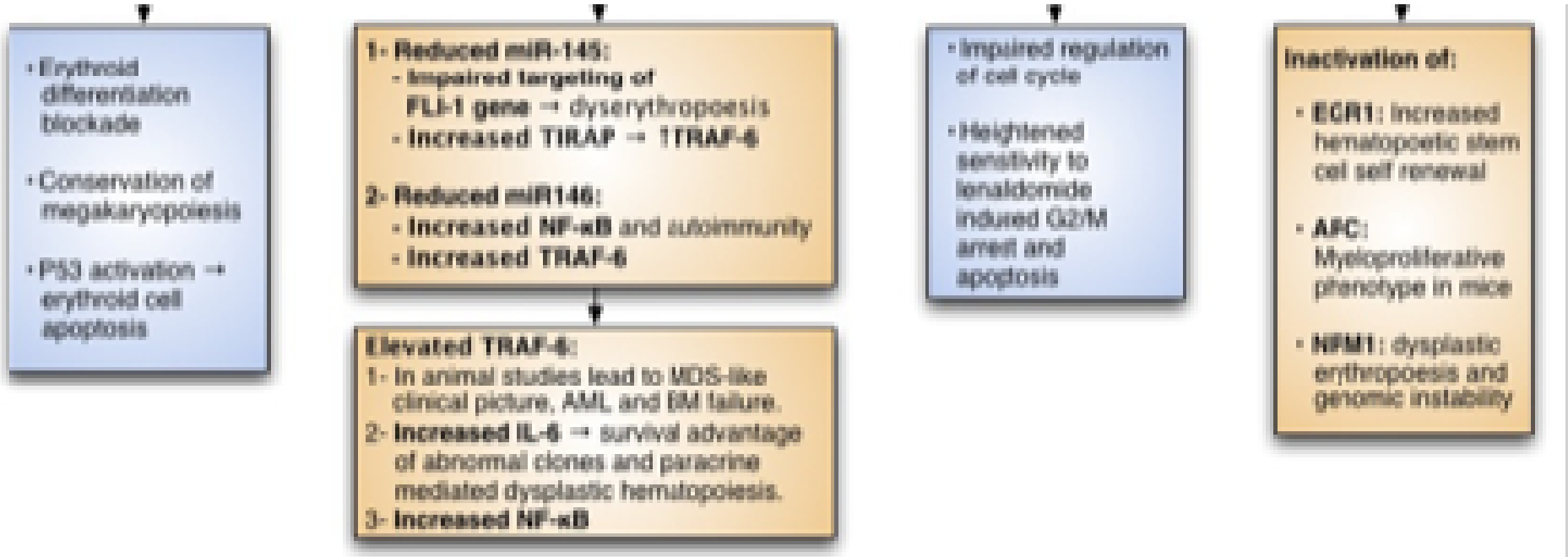
Chromosomal abnormality involving 5q is present in 20% to 30% of all MDS.
Patients who have deletion 5q can be grouped into 3 distinct categories with variable prognoses.

[1]Giagounidis A et al, Ann Hematol 2005;84:569-571
[2]Adapted from Vardiman, J. Hematology 2004, ASH MDS Education Session
[3]Olney HJ, Le Beau MM. *Best Practices & Research Clinical Haematology* 2001; 14(3):479-495
[4]Cortes JE, et. al, *Cancer Management: A Multidisciplinary Approach*, 9th ed., 2005:825-842

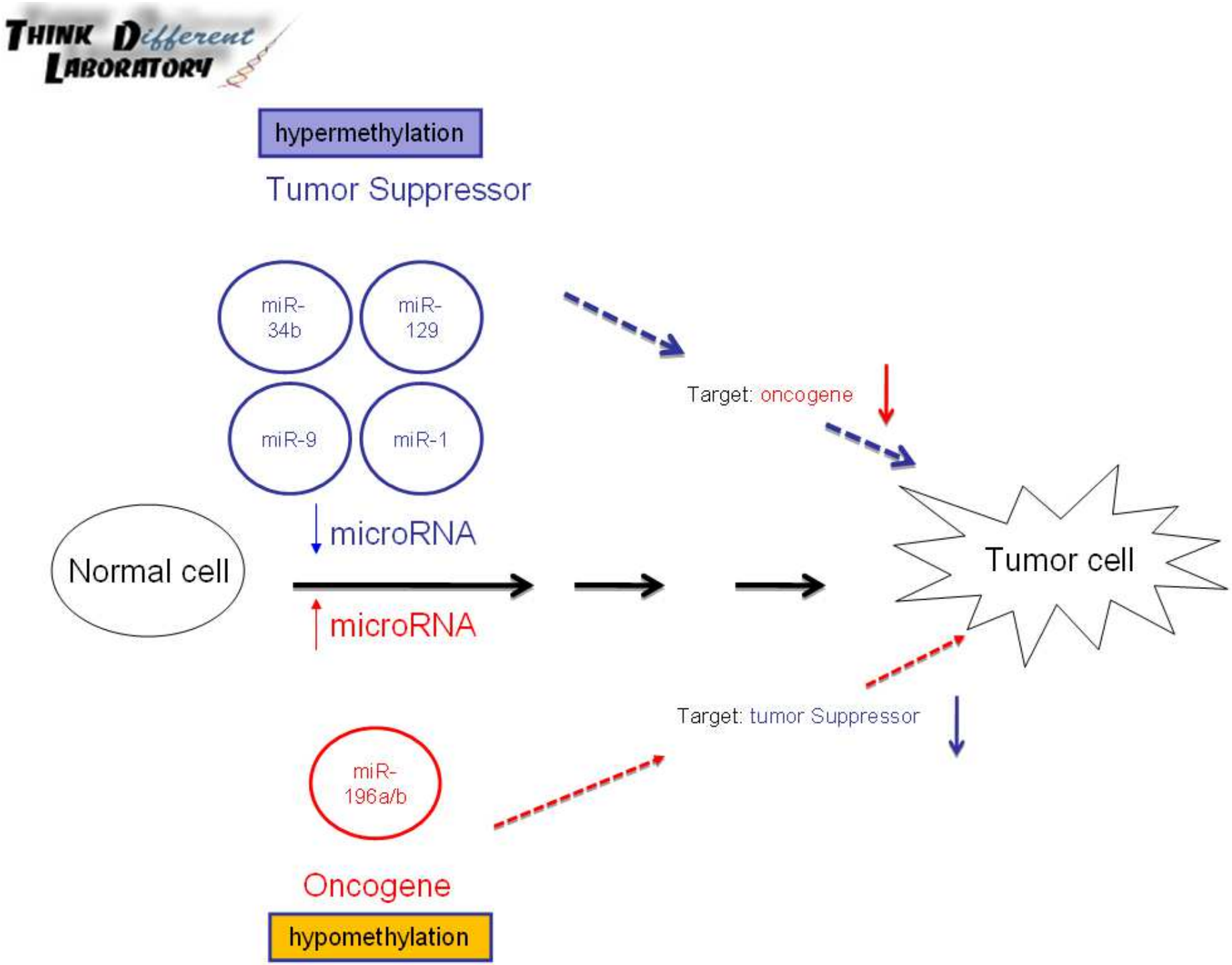
Del(5q) MDS is a Ribosomopathy



Importance of micro-RNA in the del(5q-) and p53 Apoptosis



Critical Break Regions in del(5) MDS and subsequent pathogenetic events


THINK Different LABORATORY
hypermethylation
Tumor Suppressor
miR-34b
miR-129
miR-9
miR-1
microRNA
Normal cell
microRNA
Target: oncogene
Tumor cell
Target: tumor Suppressor
miR-196a/b
Oncogene
hypomethylation

MDS-002/003: LEN Treat – Erythroid Response at 24 wk (Final Report)

List AF et al. *J Clin Oncol.* 2005;23(suppl 16S):2s [abstract 5]
List AF et al. *N Engl J Med* 2006;355>1456-1465]

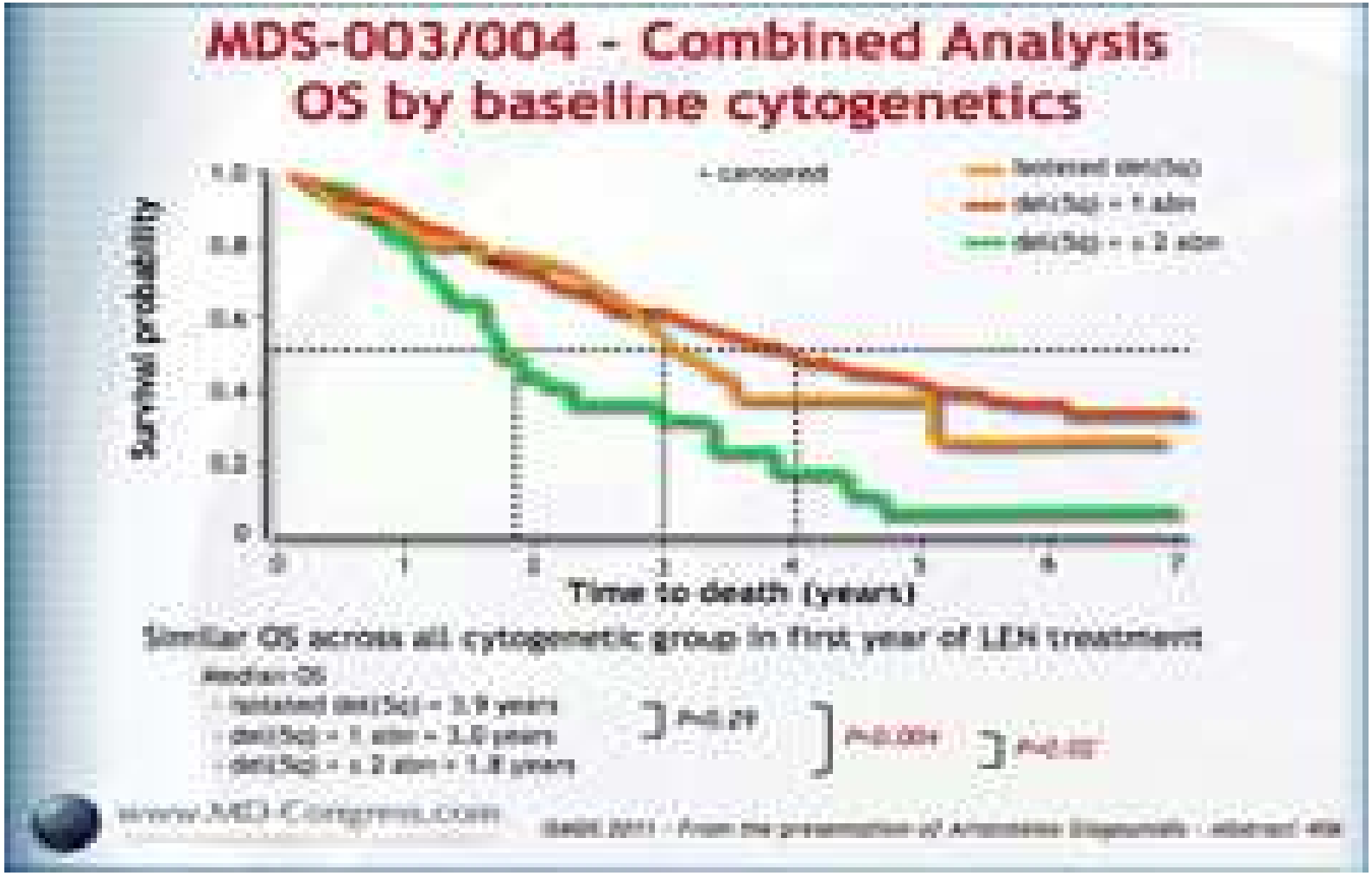
MDS-003/002: Frequency of Cytogenetic Response to LEN

Complexity	Pts Evaluable	Cytogenetic Response	CCR
Isolated 5q- n(%)	64	49 (77)	29(45)
5q- + 1 abn- n(%)	15	10 (67)	6 (40)
Complex (>3 abn)- n(%)	6	3 (50)	3 (50)
Non-5q- group*	20	9 (45%)	n.a.

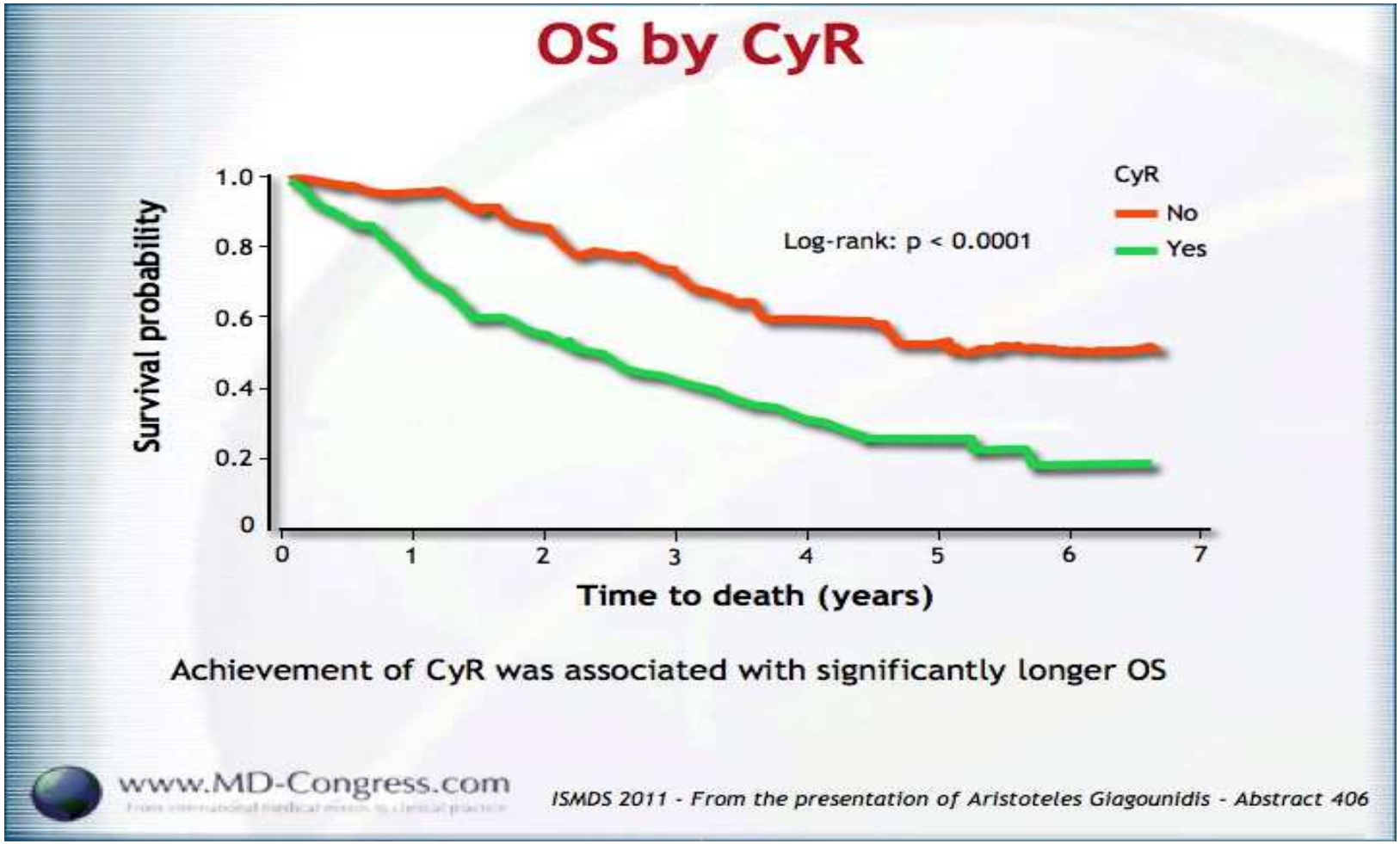
* Data on file. Celgene Corporation

List AF et al. *N Engl J Med* 2006;355>1456-1465]

A pooled analysis of MDS 003/004



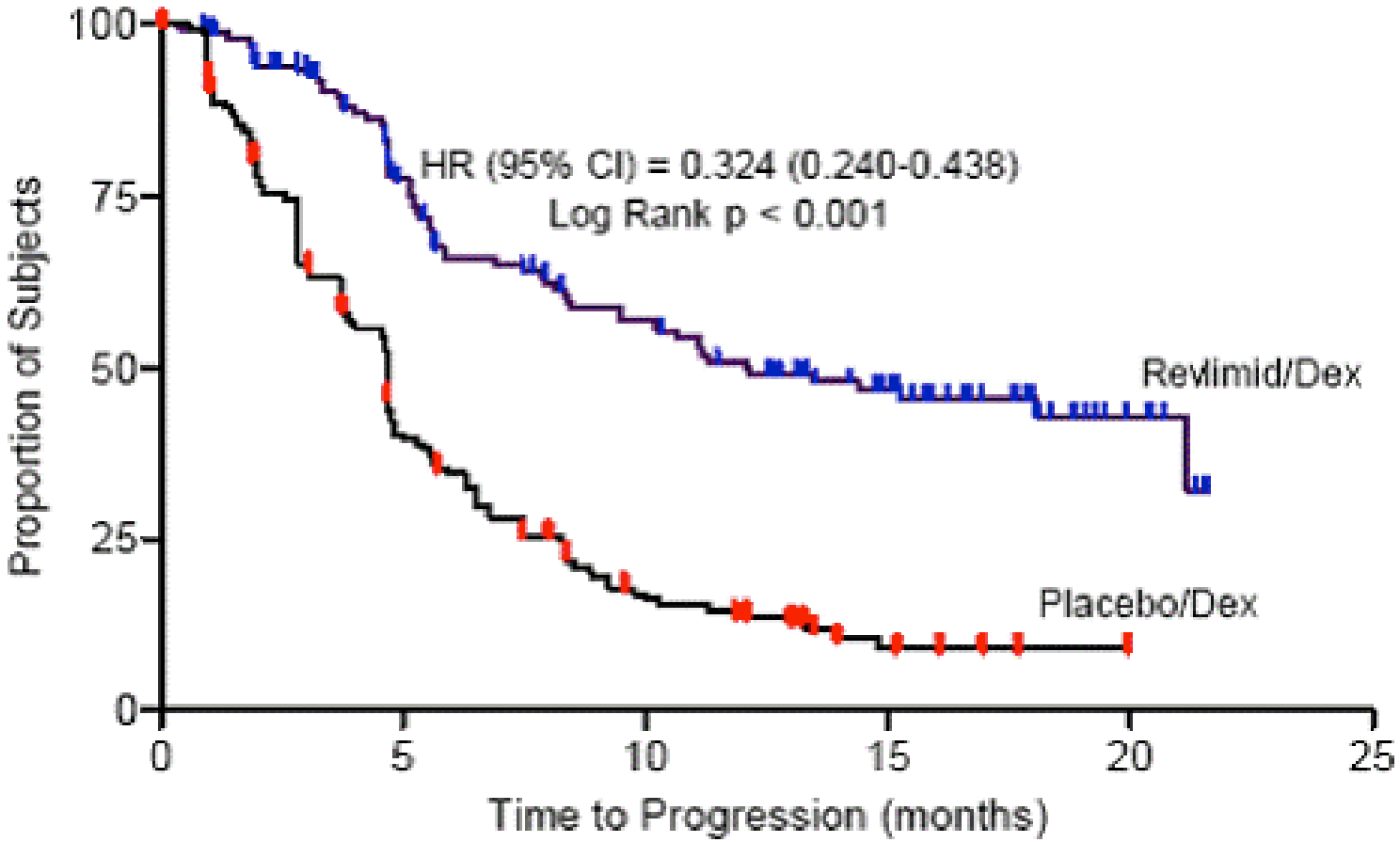
Overall Survival of del(5q) MDS patients by Cytogenetic Response



TP53 suppression promotes erythropoiesis in del(5q) MDS, a targeted therapeutic strategy in lenalidomide-resistant patients

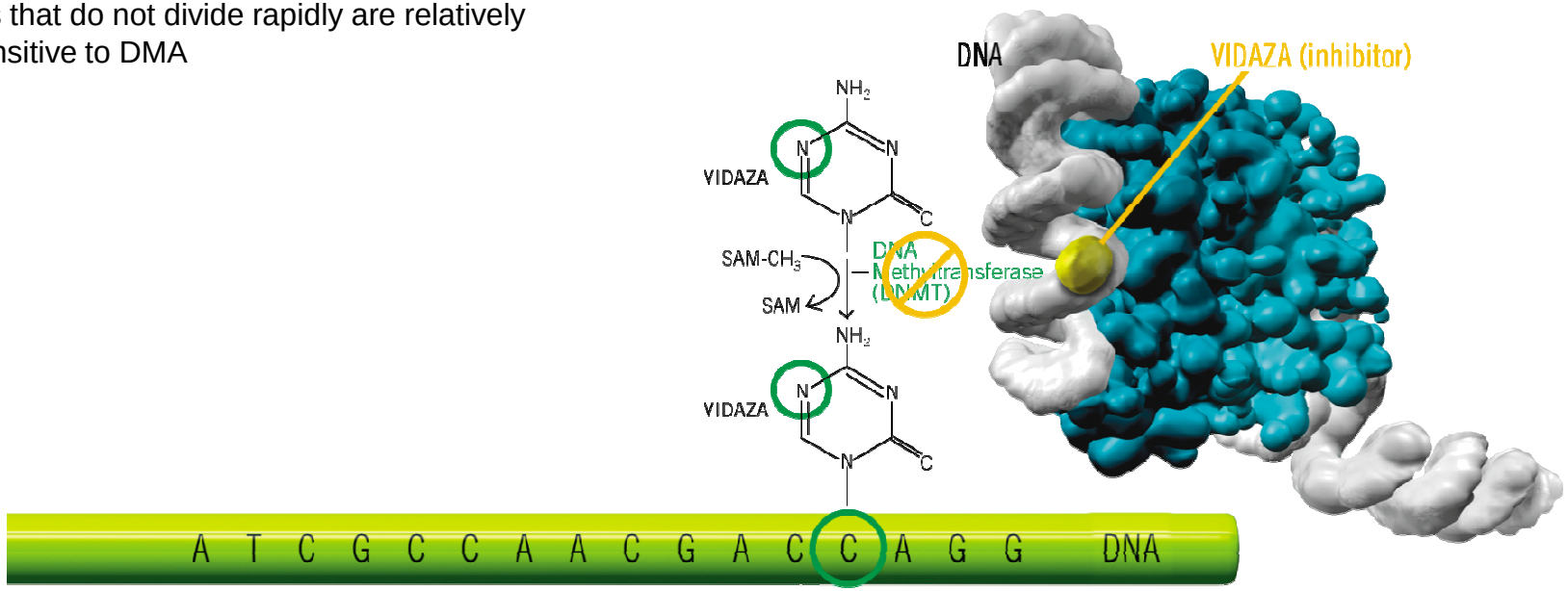
- Anemia characteristic of del(5q) MDS arises from ribosomal protein insufficiency, resulting in erythroid-specific activation of p53.
- 8 lower-risk, LEN-refractory del(5q) MDS patients treated with LEN/DEX, a glucocorticoid receptor-dependent antagonist of p53, transfusion independence was restored in 5/8 patients, with *in vivo* expansion of erythroid precursors without clonal suppression.
- Inhibition of p53 may be a unique therapeutic strategy in patients with lenalidomide-resistant del(5q) MDS.

Overall Survival between Len/Dex vs Placebo/Dex



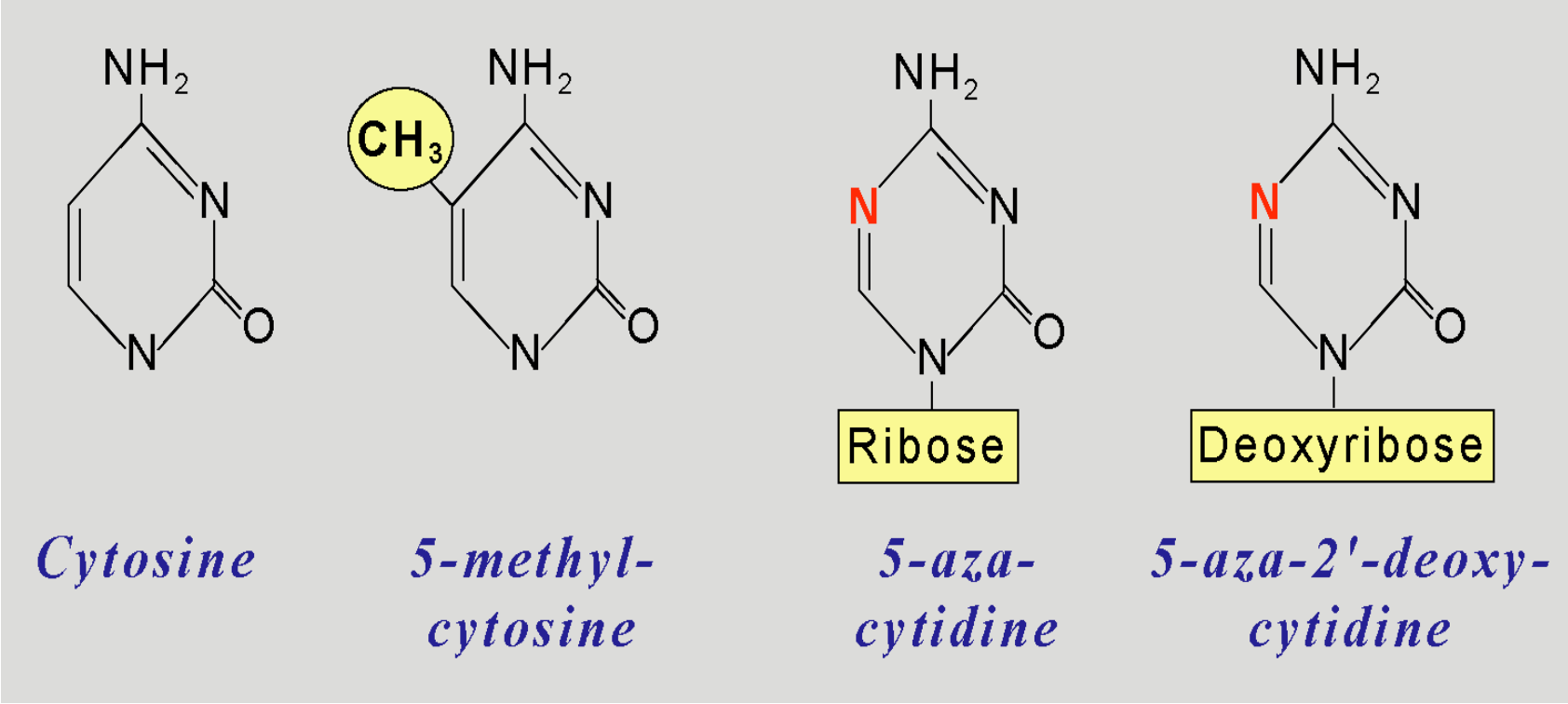
Mechanism of Action of DMA in DNA or RNA function

- By inhibiting hypermethylation, DMA may restore normal function, or expression, to genes that are critical for cell differentiation and proliferation.
- DMA a also causes the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms.
- Cells that do not divide rapidly are relatively insensitive to DMA

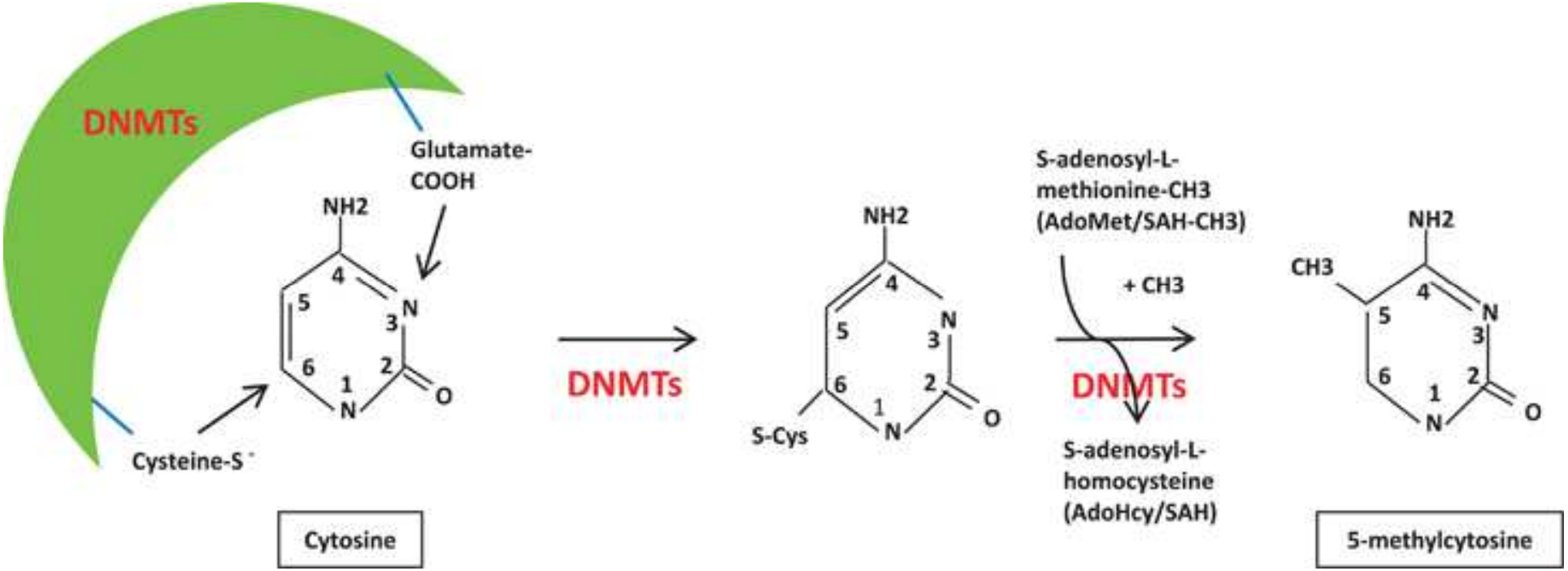


Hypomethylating Cytosine Analogs

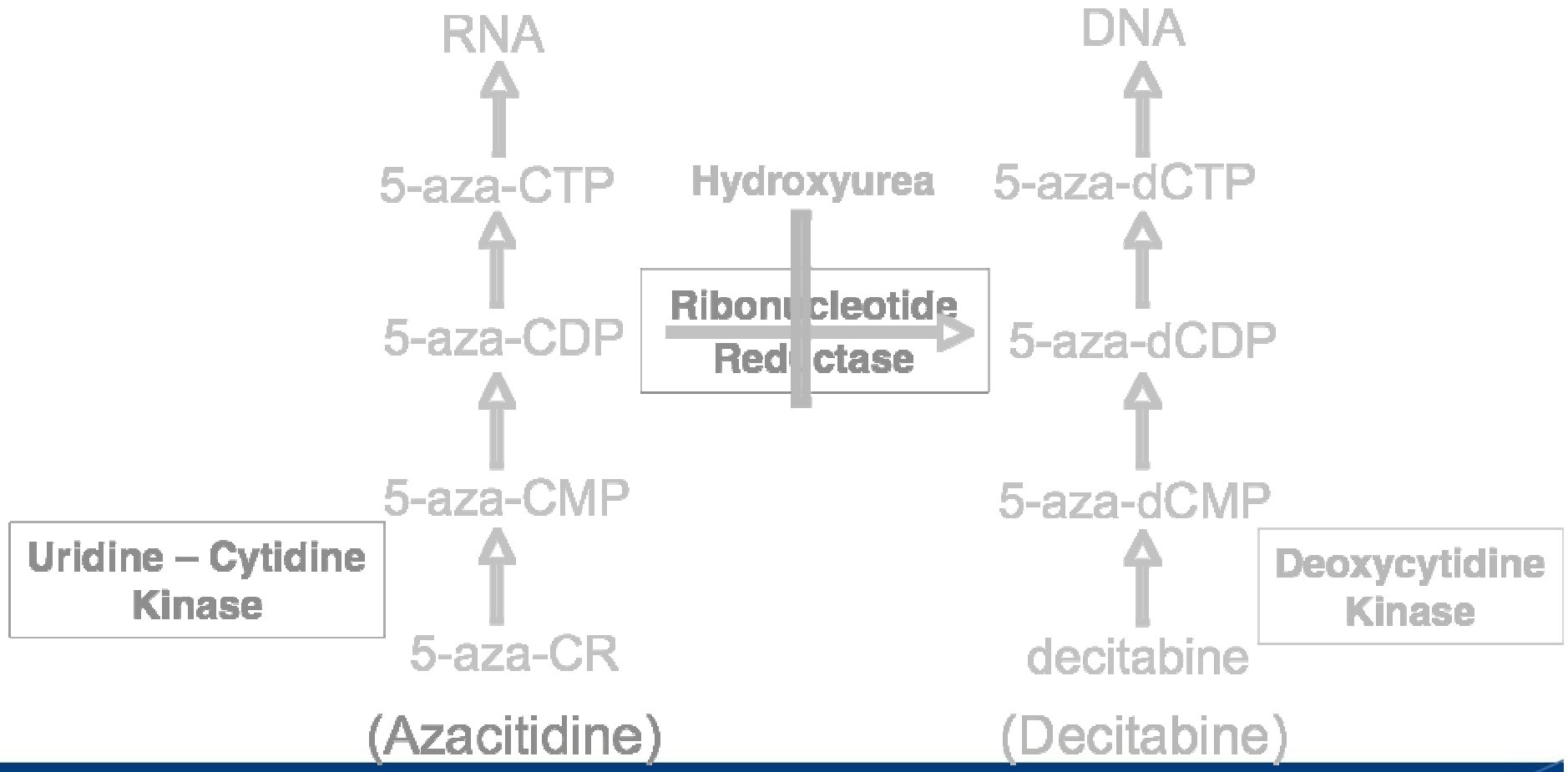
Leone G, et al. *Haematologica.* 2002;87:1324-1341



Mechanism of Action of DMA in DNA or RNA function

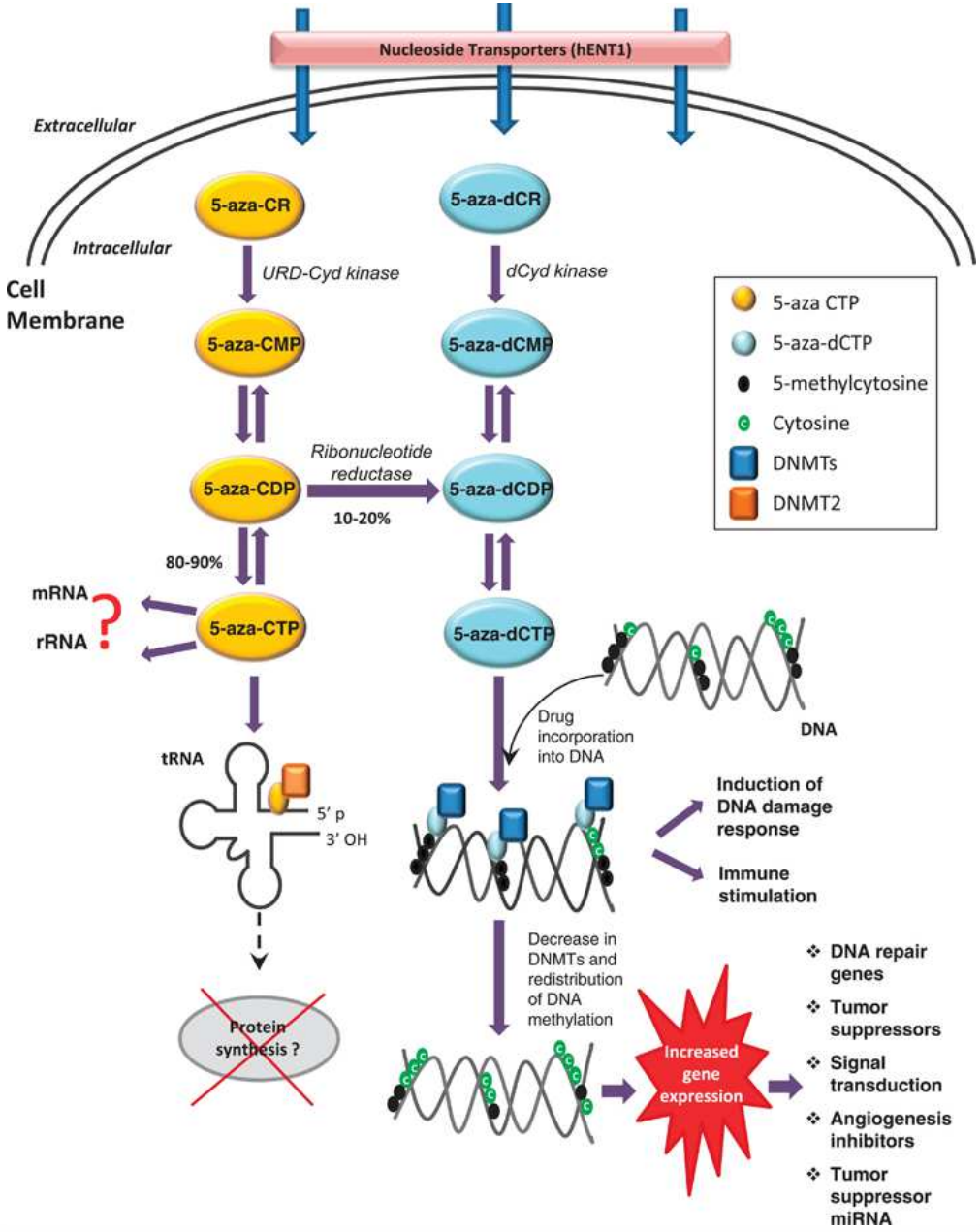


Hypomethylating Agents and Hydroxyurea

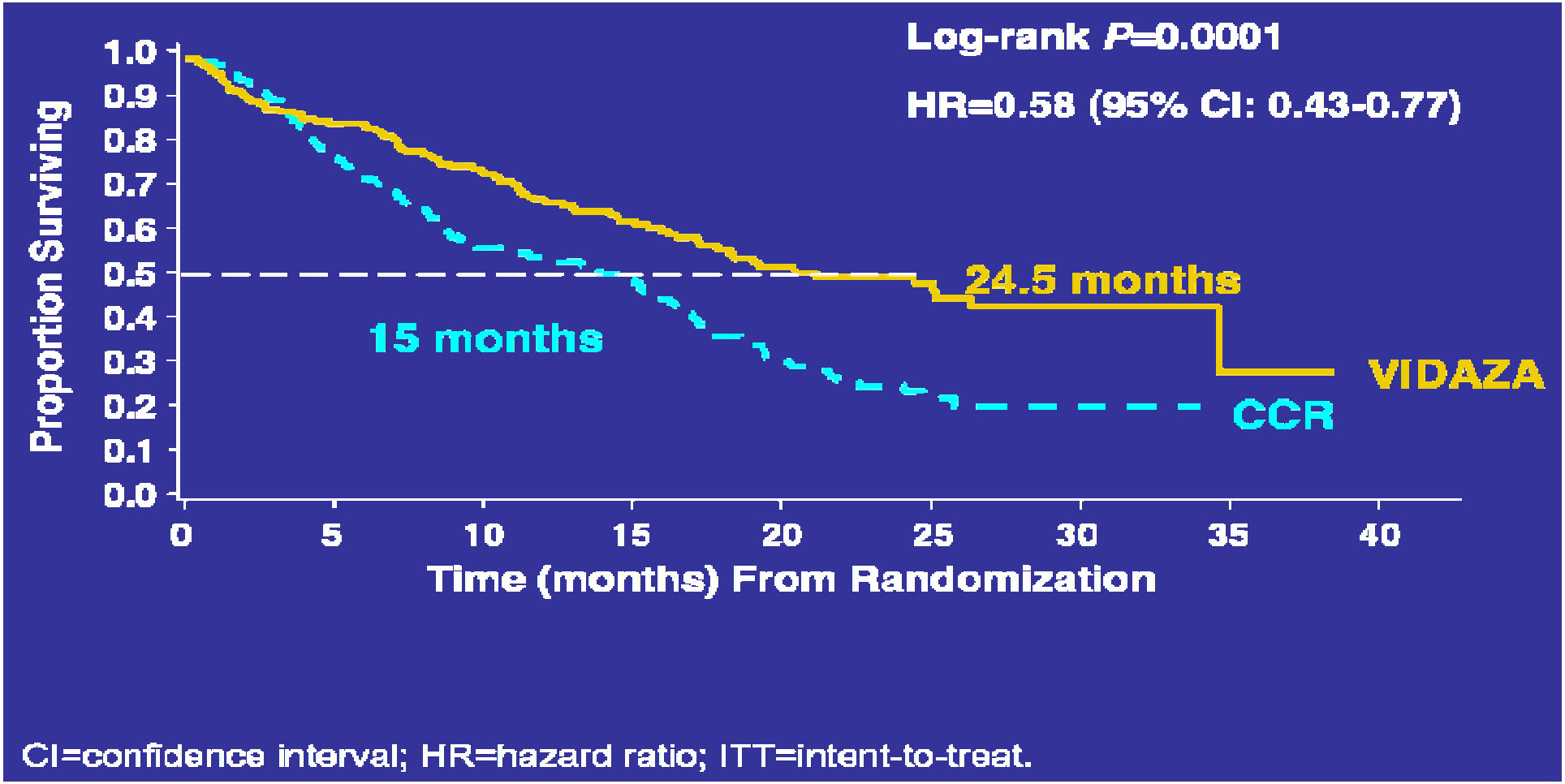


Attadia V. *Leukemia*. 1993;7:9-16.

Mechanism of Action of DMA in DNA or RNA function

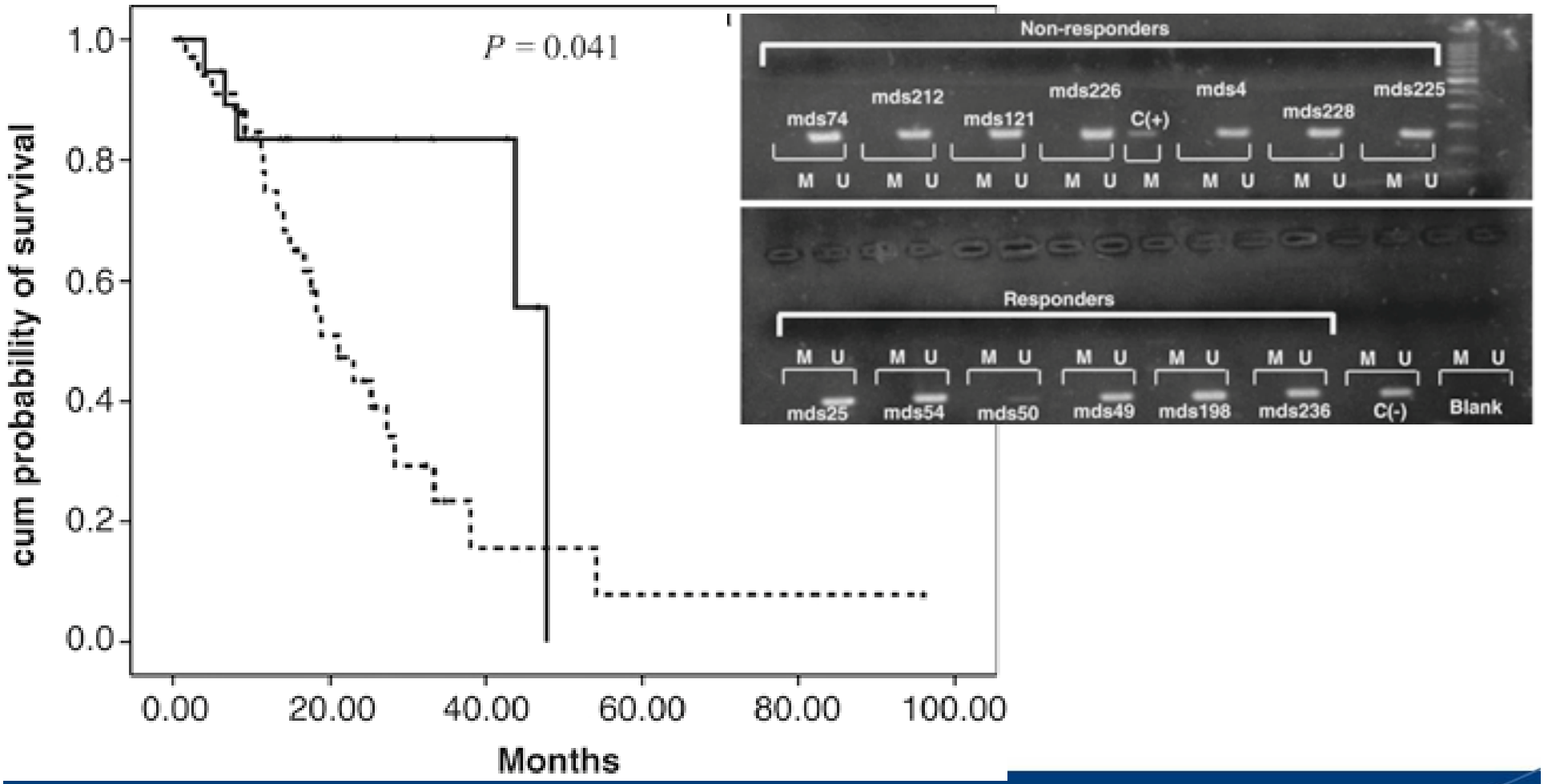


AZA-001 Trial: VIDAZA® Significantly Improves Overall Survival (OS)



CI=confidence interval; HR=hazard ratio; ITT=intent-to-treat.

Overall Survival of Aza-Rx MDS patients by UCK1 expression



Personalized Approach to Diagnosis & Treatment in MDS & AML

- Research in the molecular mechanism of malignancy and in leukemias have opened up a new personalized approach to complex diseases in cancer.
- A diagnosis cannot determine appropriate therapy since there are multiple variations within a disease.
- Hematologic malignancy is an ideal field for this approach since the blood and bone marrow is readily accessible for testing.
- Clinical trials and cooperative groups using databases of multiple countries and cancer trial groups have improved our understanding of the clinical significance of cytogenetics and genomic markers.
- The future looks bright in solving and curing this formidable disease.